Federation Proceedings

VOLUME 14 *June 1955* NUMBER 2

NOTES ON THE THIRTY-NINTH ANNUAL MEETING OF THE FEDERATION

San Francisco, April 11–15, 1955

The 39th Annual Meeting April 11–15, 1955, in San Francisco, California, was the first West Coast Meeting of the Federation. Registered attendance included 1226 members of Constituent Societies, 1986 non-member scientists, 332 graduate students, 291 wives of attending scientists, 470 exhibitors and 32 press representatives, a total of 4337.

Scientific sessions were held in the Civic Auditorium, Masonic Temple, Public Health Building and Hotel Whitcomb, April 12–15. In all, 1354 papers were presented in 130 sessions with an additional 228 papers listed in the program as read by title. Eight of these were Inter-Society Sessions in which papers from each society were presented on the topics of atherosclerosis, blood clotting and radiation effects. Organized on an experimental basis for the first time during the 1955 Meetings, these sessions proved so satisfactory that they will probably be continued in programs of future meetings. Two teaching sessions, one a Physiology panel discussing aims and methods of laboratory instruction and the other a Pathology panel considering the report of the Second A.A.M.C. Teaching Institute of Pathology, Microbiology, Genetics and Immunology, were presented. In addition three Biochemistry symposia on Amino Acid Metabolism, Biochemistry of Disease and Cholesterol Metabolism, a Pharmacology symposium on the Nature of Microbial Resistance to Drugs and a Nutrition Symposium on Lipids in Nutrition were presented during the week. A special session, under the chairmanship of Dr. C. R. Treadwell, was devoted to presentation of six motion pictures. The Poultry Nutrition Conference and a round table discussion on Rare Earths in Biochemical and Medical Research, sponsored by the Oak Ridge Institute of Nuclear Studies, were special meetings scheduled April 11 and 14 respectively.

The American Society for Experimental Pathology, senior society at the Meeting, sponsored the program of the Joint Session, Tuesday evening, April 13. Dr. D. M. Angevine, Past-President of the Pathology Society and Chairman of the Federation Board, presided. The three papers presented by Dr. K. R. Porter, Dr. G. H. Hogeboom and Dr. F. B. Bang on the general topic "Integration of Cell Structure and Function," will be published in this and future issues of FEDERATION PROCEEDINGS.

The Federation Board and the six Society Councils met beginning April 11, and Society Business Meetings were held during the week. A list of officers elected for the coming year appears below. In addition, Editorial Boards of the journals of the Constituent Societies, the Society for Experimental Biology and Medicine and the RES Society as well as a number of special committees used the facilities of the Meetings for conferences.

The Pharmacology, Pathology and Nutrition Societies held their annual banquets; Pharmacology and Pathology each had scientific programs following dinner, while the presentation of awards followed the Nutrition Dinner. The Biochemistry and Nutrition Societies sponsored a Smoker following the Joint Session Tuesday evening. Special-interest groups met for dinner followed by scientific programs and discussion. Numerous reunions and social gatherings met for breakfasts, luncheons, dinners and during the evening hours.

A Ladies Committee headed by Mrs. John J. Sampson greeted the wives of members and non-member scientists, and assisted them in becoming acquainted with each other and the San Francisco Bay Area. Beginning with a tour of the San Francisco Art Gallery followed by tea on Monday, April 11, the ladies visited Stanford University and points of interest near Palo Alto, the Sonoma Valley Mission and wineries, the shops in San Francisco and Chinatown during the week. The gracious efforts of the Ladies Hospitality Committee and their program of interesting activities made the San Francisco Meeting a memorable one for guests of the Federation.

On Monday, April 11, the laboratories and science departments of the University of California held open house for the registrants at the Federation Meetings. The Life Sciences Building, Donner Laboratory, Biochemistry-Virus Building, Home Economics Building and the Western Regional Research Laboratories of the U. S. Department of Agriculture were open for inspection,

and a hospitality and meeting center was located at Alumni House. The Federation is indebted to Dr. D. M. Greenberg, the department and laboratory heads, their assistants and students for arranging transportation, guides and hostesses, and to Chancellor Clark Kerr for affording this opportunity to visit the remarkable scientific facilities of the University.

More than 100 exhibits by 87 industrial firms, members and their associates were located in the Main Arena of the Civic Auditorium. The industrial exhibits presented displays of books, apparatus and equipment, pharmaceuticals, chemicals and food products. Institutional displays depicted the activities of the foundations and societies represented, while members and their associates took advantage of the opportunity for visual presentation of their work.

The Federation is indebted to the Committee, Friends of Science of California, for assistance in underwriting the expenses of the 1955 Meetings. Dr. John H. Lawrence, General Chairman, Mr. George D. Smith, Executive Chairman, and a committee of forty California scientists and business men contacted industrial organizations, which might contribute to the meetings, and specialty firms on the West Coast to inform the latter of the opportunity to exhibit at the Federation Meetings. Their efforts resulted in a better acquaintance with industrial firms operating in the fields represented by the Federation. The contributions of the following firms are gratefully acknowledged:

Don Baxter, Inc., Glendale, California
Cutter Foundation and Laboratories, Berkeley, California
Distillation Products Industries, Rochester, New York
Rexall Drug, Inc., Los Angeles, California
Riker Laboratories, Inc., Los Angeles, California
S & W Fine Foods, Inc., San Francisco, California
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FUTURE MEETINGS

The next annual meeting of the Federation will be in Atlantic City, New Jersey, April 15–20, 1956. The 1957 Meeting will be April 14–20 in Chicago; the 1958 Meeting will be in Philadelphia, April 13–19; the 1959 Meeting will be in Atlantic City, April 12–18 and the 1960 Meeting will be in Chicago, April 10–16.

JOINT SESSION OF THE FEDERATION

San Francisco, California, April 12, 1955

Integration of Cell Structure and Function

Chairman: D. MURRAY ANGEVINE

PATHOLOGY OF THE CELL INFECTED WITH VIRUSES—MORPHOLOGICAL AND BIOCHEMICAL ASPECTS[1]

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A NATURAL sequel to discussion of the structure and enzyme constitution of the normal cell is a review of changes produced in the cell by viruses. This review will consider mainly vertebrate cells and their viruses. The parasitic dependency of some viruses on their host cells is so complete that it is risky to speak of action and reaction within the cell. Cell morphology may change dramatically during the course of infection, but how much of this change is the virus itself and how much is the cell's reaction to it is not clear at this time.

Until recently the pathologist who studied viral lesions used the classical methods of light microscopy and studied mostly fixed specimens. Further progress in the study of abnormal cells was limited within this framework. Dr. Cowdry in 1928 summarized contemporary studies of cell pathology (1). Using these as a basis, viral lesions were classified as those producing intracytoplasmic inclusions and those producing different kinds of intranuclear inclusions (2). Ludford has further described pathological changes, especially in living cells, and has included some recent phase microscopy (3).

The complex of new techniques applied to the study of the abnormal cell calls for a re-evaluation of our knowledge and of the generalizations based on that knowledge. However, we probably do not yet have enough information to warrant a differential classification of viruses based on the types of lesions produced in the cell. Indeed, it already seems clear that two strains of the same virus species may produce different types of lesions; and the same virus may produce different lesions on two different cell types. Our discussion will start with a review of methods of studying the infected cell.

Two techniques are of recent importance in the study of sick cells. The first is phase microscopy, in which Gey has pioneered in this country (4), as has Frederic in France (5) and Ludford in England (3). The other is electron microscopy, which was at first applied to cells in tissue culture preparations and more recently to thin sections of cells. Dr. Porter has summarized the observations in normal cell structure in the preceding discussion (6).

There have been scattered attempts to apply histochemical methods to infected cells. These include the application of analyses for desoxyribonucleic acid and ribonucleic acid by the classical methods of ultraviolet absorption. Very recently, electrophysiological methods have been used in a study of pathological cell function.

LIVING CELLS—TISSUE CULTURE

Phase microscopy has made it possible to study cell structure in living cells at resolutions comparable to those used on fixed and stained cells. Because of the special demands of phase micros-

[1] None of the illustrations in this lecture have been previously published and a number of the sections were cut especially for this study. This work, as in the case of previous publications, has been supported by grants from the National Cancer Institute (C1230) and the National Microbiological Institute (E135) of the Public Health Service.

copy these studies have been almost exclusively made on cells in culture. In many cases this is of great advantage, in that a particular uniform cell line may be studied under standardized conditions.

The degree to which changes seen in these tissue culture cells are similar to the changes produced by the agent in the host is not known. However, one should remember that the extreme thinness of cells in tissue culture is no greater than that of endothelial cells lining the capillaries of the alveoli of the lung as they are now seen in the electron microscope.

The classical studies of Bland and Canti (7) on psittacosis and of Bland and Robinow (8) on vaccinia virus were carried out in tissue culture cells which were observed to develop their inclusions in the living cell. Careful fixation and staining of the same cell which had been studied in the living allowed them to follow the development of the virus lesion, and to determine finally that these two types of inclusions consisted of 'colonies' of virus particles. Similar studies with phase microscopy would be extremely valuable today. By these means it would be possible to determine something about the changes in mitochondria occurring during the initiation of infection, and to follow the development of the changes in the nucleus with herpes virus. Recent emphasis on the 'cytopathogenic' activity of viruses has led to more detailed studies of the effect of some of the destructive viruses on cell structure. In collaboration with Dr. Gey, we were able to show with phase microscopy that one of the first definite changes in cells infected with the virus of Eastern equine encephalitis was destruction of the peripheral thin spread of the cytoplasm, leaving a pattern of long, irregular projections (9). Subsequently the cytoplasm disintegrated but the nuclei were left relatively intact. Analysis of the destruction of a human cell line by poliomyelitis showed the same process taking place but leaving nuclear remnants behind (10). Electron microscope studies of these same cell-virus infections led to the suggestion that a mechanism for the escape of viruses from cells without immediate destruction of the cell was implicit in the formation of these irregular and disintegrating cell extensions (11). The subsequent determination by Dulbecco (12) that infectious virus is gradually released from cells agrees with these morphological data. Recently, Barski and collaborators have carried out similar studies on two types of poliomyelitis virus on human fibroblasts, and have described the formation of a large inclusion within the cytoplasm which forces the nucleus to the periphery of the cell. Concurrent with this is a vacuolization of the cytoplasm of the infected cells, which is considered a possible mechanism for the release of virus (13). The central lesion is similar to that described by Ludford (3) in some normal and malignant cells as a degeneration phenomenon, so it may not represent a specific viral lesion.

Flewett and Challice (14) in studies of living epithelial cells infected by fowl plague have noted an increase in the size of the nucleolus. The relationship of this apparent change to the infection itself is not clear.

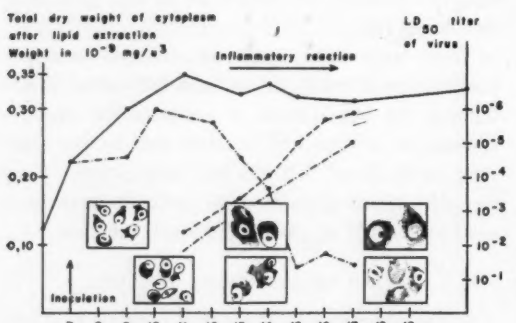

FIG. 1. Diagram of changes in cell nucleic acid content and virus titer in chick embryos infected with rabies virus. Modified from Sourander (17).

ULTRAVIOLET ABSORPTION STUDIES

The first serious study of nucleo-protein metabolism in relation to virus reproduction was carried out by Hyden in Casperson's laboratory (15). This study was essentially a survey of the variety of changes which may occur in infected cells, and included a series of photographs taken at 2600 angstroms of *Molluscum contagiosum*, a human pox virus. He showed that strongly absorbing material occurred in the cytoplasm in the area corresponding to the inclusions. Feulgen stains showed these to consist of DNA. Other cells infected with virus were studied for protein

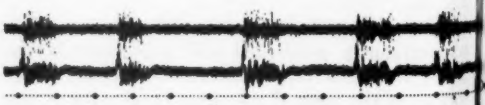

FIG. 2. Bursts of spontaneous activity discharged from sympathetic ganglion of rat infected with pseudorabies virus.

metabolism by ultraviolet photography. A greater polynucleotide absorption seemed apparent later in the infection. He concluded that the data indicated that viruses act "as parasites upon the nucleoprotein forming part of the host cells."

Similar findings have recently been reported on infections of mouse muscle cells with Coxsackie virus (16). Again an increase in the protein was indicated and degenerative changes, particularly of the cytoplasm. The conclusion was that the virus affected the protein synthesis of the host cell.

The most complete study on one cell system is that of Sourander (17) whose findings are summarized in the accompanying chart (fig. 1).

In this one may see that with the development of rabies virus in the central nervous system of the chick embryo, certain specific changes accompany the virus infection. In contrast to the uninfected cells, which increase in mass until day 9 of incubation age and then remain fairly constant, the cellular mass of infected cells dropped from the 5th day after inoculation (day 12 of incubation) to $\frac{1}{10}$ that of the uninfected by day 15 or 8 days of infection. The cytoplasmic content of ribonucleic acid of control cells remained high, and that of infected cells dropped concomitantly with the decrease in mass of the cell. By 10 days after inoculation the nerve cells were practically empty of ribonucleic acid. There was a good correlation between the amount of virus in the brain and these changes. The inflammatory response, however, occurred several days after the changes in cell mass and RNA content. Sourander concludes that his data support the thesis that the virus acts on the nucleoprotein apparatus of the cell. Since, however, this 'apparatus' is an essential part of the cell's metabolism, it seems difficult to know whether the changes described in all of these different studies are primary, or whether the changes produced are merely those which would be expected to occur in a cell which was dying for reasons other than virus infection. Lysis of the substance following section of the axone of the nerve is well known.

ELECTRON MICROSCOPY

Two general methods for preparation have been used for the study of infected cells. The first takes advantage of the thin spread of cells grown in tissue culture on formvar-coated glass. Such cells may be infected with virus, harvested at any subsequent interval and then studied as whole mounts. The residual skeleton of the cell after disintegration may be seen as well as the initial changes produced by the virus in the periphery of the cell. Cell lines may be studied easily and under controlled culture conditions. Such tissue culture cells may also be sectioned and the effect of the virus observed deeper in the cell. This procedure complements the first but does not replace it, because embedding and sectioning entail some mechanical damage and because one may not see the full spread of virus infection. Both methods need to be used in the study of tissue culture preparations. An amazing succession of improved methods of thin sections of cells have made it possible to achieve resolutions up to 100× those of the light microscope. Several groups in this country are using these methods in studying virus infections.

BIOCHEMICAL METHODS

A variety of studies of the changes produced in tissue following virus infection have been summarized recently (19). In a particularly careful study carried out by Margaret Smith and Kun (20), the changes in the chick chorio-allantoic membrane were followed. It was found that with a variety of viruses, including myxoma, fibroma, Rous sarcoma, swine influenza, vaccinia and herpes, there was an increase in anaerobic hexdoiphosphate breakdown. This change in enzyme activity did not occur either after inactivated virus was inoculated or after marked inflammation was produced by sterile celite. The changes were part of an increased glycolysis and occurred in the absence of respiration.

The difficulty of applying this type of study to an understanding of the changes in individual cells lies in the fact that a tissue such as the chorio-allantoic membrane consists of a variety of cell types in various stages of cellular disease.

TABLE 1. AREAS OF CELL AFFECTED BY VIRUSES

	Normal	Pathological
Surface	Microvilli	Long filaments
	Cilia	Breakdown and release
	Excretory droplets	?
Cytoplasm	Mitochondria	Distended and disintegrated
	Endoplasmic reticulum (ergastoplasm)	Destroyed
Nucleus	Nucleolus occasionally 'coiled'	Virus in nucleus Nucleolus abnormally coiled or destroyed

Perhaps the greatest value of such studies on tissue will be apparent when the changes produced in uniform cell populations may be compared with changes produced in fixed tissue.

An entirely different approach to the study of abnormal cell function induced by viruses is that of Dempsher *et al.* (21). They have used the techniques of electrophysiology to follow the function of infected cells. By placing electrodes on the preganglionic and postganglionic nerves of the superior cervical ganglia of a rat they were able to pick up the discharges from the diseased

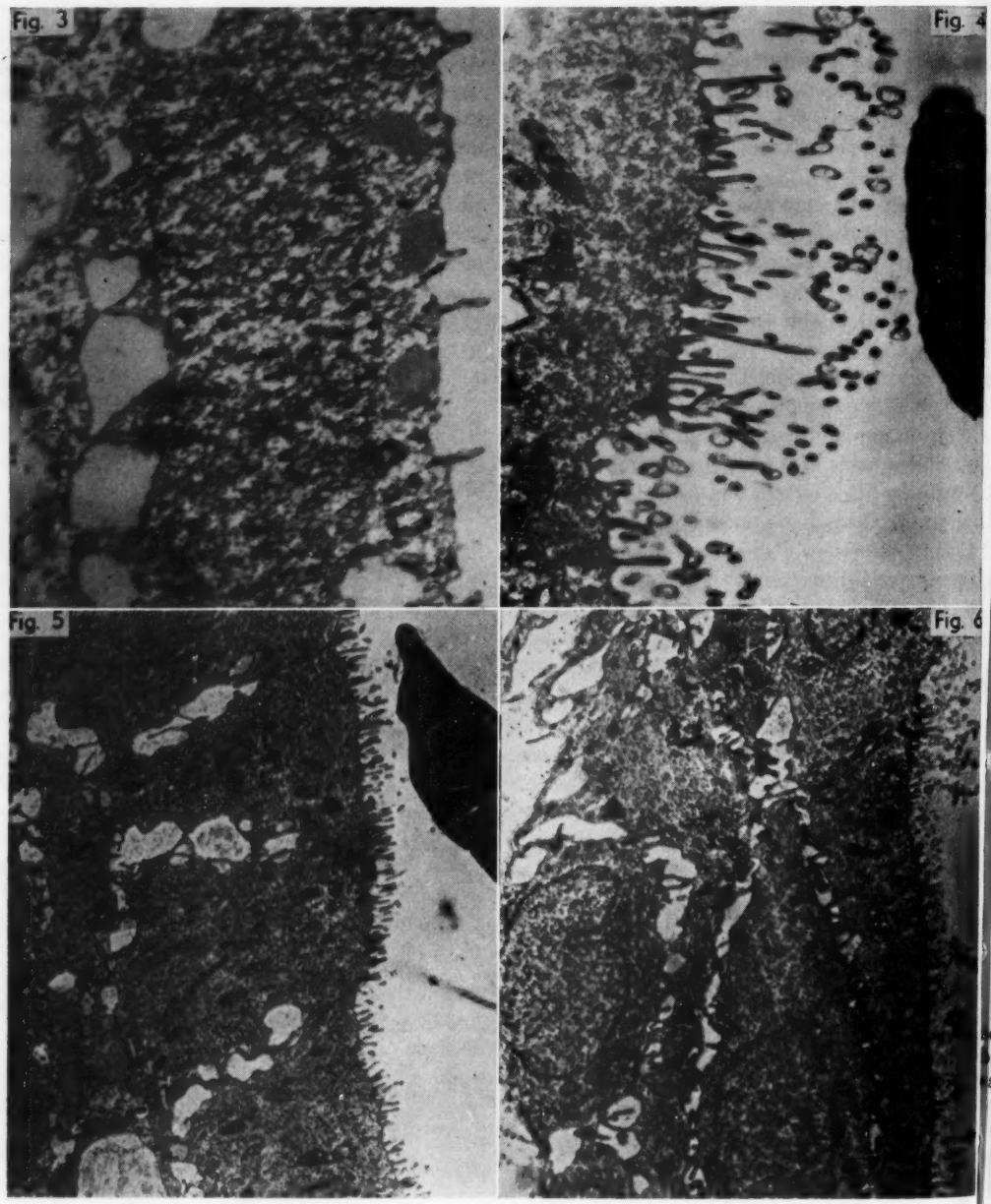

FIG. 3. Normal allantoic cell from 15-day chick chorioallantoic membrane.

FIG. 4. Newcastle infection with avirulent strain of 15-day-old embryo–infection initiated 4 day previously.

FIG. 5. Hyperplasia of allantoic membrane following 4 days of infection with avirulent strain of Newcastle virus.

FIG. 6. Hyperplasia of allantoic membrane following 4 days of infection with influenza virus

ganglia. The normal ganglia, when in the intact rat or when removed and placed in an isotonic bath, did not give rise to spontaneous discharges. In contrast, the ganglia infected with the virus of pseudorabies which has been graphically called 'mad itch', continually gave off spontaneous bursts of activity (fig. 2). The development of these bursts is closely correlated with the development of virus in the ganglion and with the pathological changes produced by the virus. The changes occur first in the part of the ganglion first reached by the virus. Impulses begin to be discharged over the postganglionic nerve 2–3 days after the inoculation of the eye of the rat with virus. Discharges over the preganglionic nerve come later, and the postganglionic discharges disappear along with the capacity for synaptic transmission through the ganglia. Addition of curare or the removal of the calcium from the fluid surrounding the infected ganglion suppresses synap-

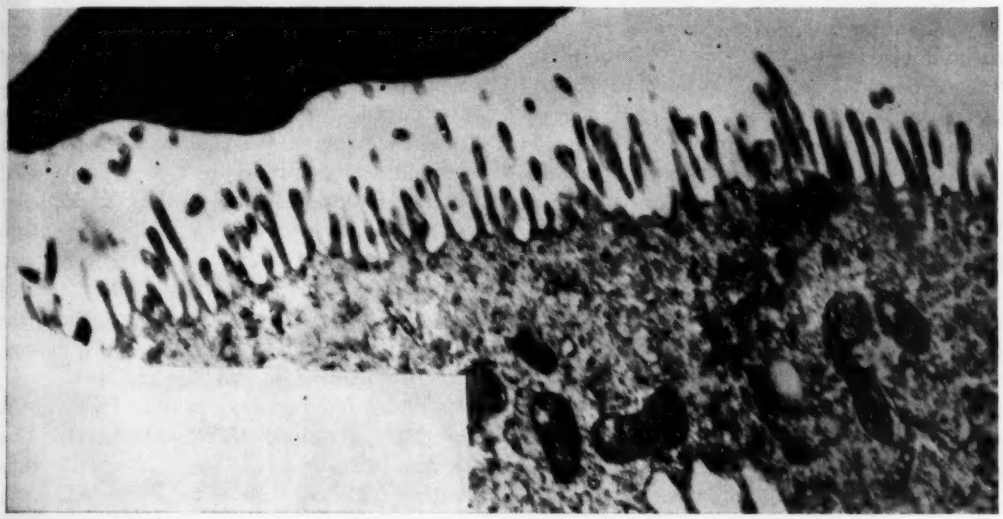

FIG. 7. Higher magnification of allantoic sac cell surface following 4-day infection with Newcastle virus.

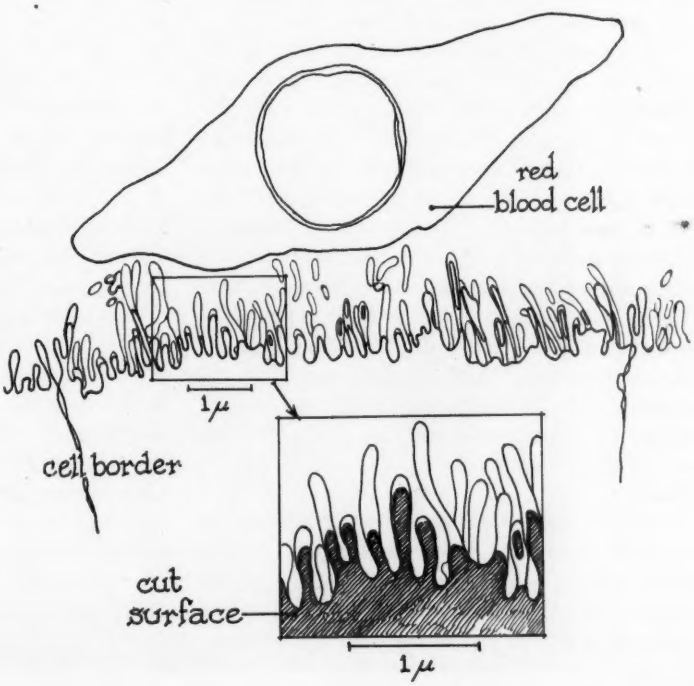

FIG. 8. Reconstruction of 9 serial sections of a chick membrane cell infected with Newcastle Disease virus.

tic transmission and spontaneous activity. From such studies it may be possible, eventually, to localize the effect of the virus. It is clear that similar studies need to be done with other virus infections of the same cell system and with the same virus in other cell systems.

PATHOLOGY OF THE CELL AS SEEN BY ELECTRON MICROSCOPY

A new classification of viruses based on their pathological effects on cells, as has been done in the past on the basis of the type of inclusion produced, is not yet useful. However, description of the type of lesion and the areas of the cell affected by the virus is worthwhile. In the accompanying table we have compared electron microscope studies of some normal structures with the changes produced by virus infection. The cells lining the allantoic sac of the chorioallantoic membrane are especially favorable for both electron microscopic and virological studies. In contrast to the cells infected by the pox viruses, these cells may be studied without the trauma introduced when the membrane is separated from the shell membrane. Thus, changes in the infected cell can be related to the virus infection. It is now clear that the normal allantoic sac cell has a variable number of small, blunt, short projections which we have called microvilli (fig. 3; 22). The function of these and the factors which influence their number and morphology have not been determined. Three viruses, all belonging to the same group (influenza, Newcastle, and fowl plague) have been studied in this area. The characteristic long filaments of influenza, which were first recognized by Mosely and Wyckoff (23) free in the allantoic fluid, can be related to the projection from the cell of many long, thin filaments (24). At the tips of the projections or filaments are spheres of the same size as the influenza virus. Tissue culture studies done by Murphy in our laboratory (25) first showed that masses of filaments and spherical particles occur intermingled, and that the spherical virus particles could frequently be found within these long filaments, sometimes breaking out of them and appearing scattered along the filaments.

Allantoic sac cells infected with certain strains of either Newcastle, fowl plague, or influenza show changes which are most marked at the surface (fig. 4). As yet, it is difficult to detect influenza virus in the cytoplasm. In addition, hyperplasia of the lining membrane frequently occurs after several days of infection (figs. 5 and 6) and this has led to the suggestion that an infected cell continues to produce virus over a period of several days without disintegration (26). Confirmatory data have been obtained by Henle (27) using accurate methods of measuring the amount of virus released into the allantoic sac, and by Schlesinger (28) on another cell system. The effect on the cell of infection with the avirulent strain of Newcastle differs somewhat from the effect of influenza virus. The filaments tend to balloon and disintegrate at the free end and the pleomorphic appearance of the virus is apparent in the less sharply differentiated segmentation of the long filamentous processes as seen in tissue culture (29). It has been frequently noted that the recently isolated strains of influenza tend to produce long, thin filaments in the allantoic sac, whereas the old strains of virus are usually spherical. Presumably the recently isolated strains are less well adapted to growth in the allantoic sac cells and thus are extruded as long, partially converted, microvilli.

In only a few instances has the effect of the virus been determined at different intervals. This was done with an active, mild Newcastle virus infection initiated with a small inoculum and followed at 24-hour intervals (26). Figure 5 shows a low power electron micrograph of the hyperplasia and tremendous surface changes taking place at 4 days. The reconstruction of the surface (fig. 7) is based on higher resolution pictures of serial sections. The effect of large amounts of fowl plague on the same cell system has been followed during the first few hours up to 12 hours (30). Under these conditions, a profusion of small projections which differ from the normal microvilli first appear at the surface of the cell 6 hours after infection. Subsequently, individual particles may be found in this surface area.

A consideration of the surface of abnormal cells calls to mind the changes produced in cells of spontaneous mammary tumors of mice. One of the necessary participants in the production of this tumor is a filterable virus known as 'mammary tumor inciter.' Several workers have found virus-like particles in the tumor cells. These virus-like particles may be either within the cytoplasm of the cell or projecting from the cells in a way essentially analogous to the pathological microvilli produced by the respiratory viruses (figs. 9 and 10). Since microvilli are commonly

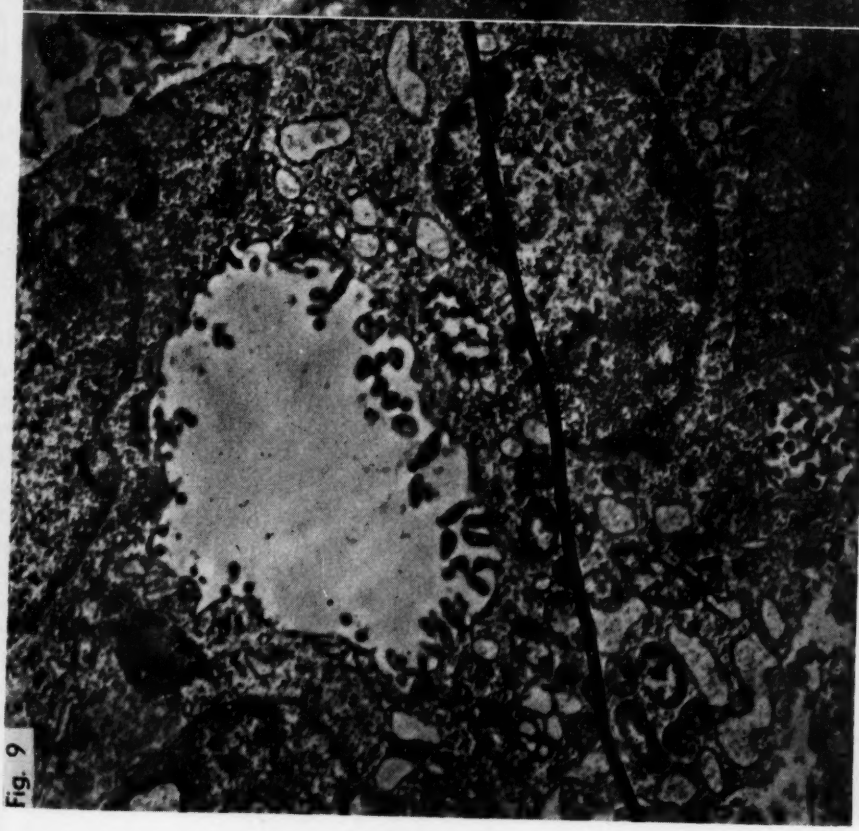

FIG. 9. Area between mammary tumor cells. Microvilli from tumor cells containing dense 'virus' particles. Spontaneous tumor in C3H mouse.
FIG. 10. Same as figure 9; from another tumor.

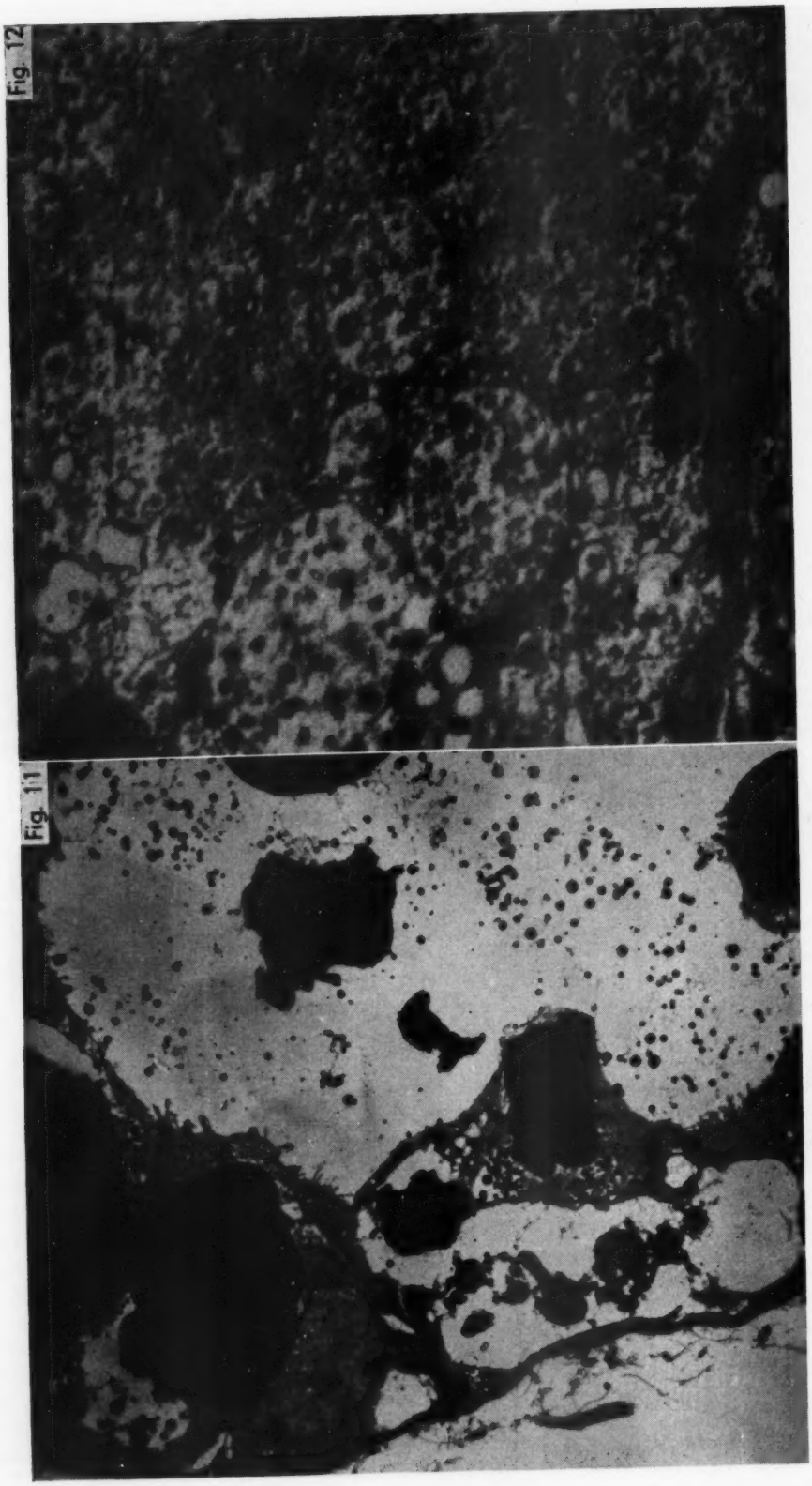

FIG. 11. Normal mammary gland, showing process of milk secretion, C3H mouse.

FIG. 12. Virus particles appearing in spontaneous mammary tumor cell, surrounding vesicles which are believed to be swollen mitochondria.

present on a variety of cells lining fluid-containing cavities, we may expect that this mechanism of escape of viruses from cells may occur in a variety of situations. Indeed, this was pointed out several years ago by Robinow when he called attention to the presence of vaccinia virus at the end of long stalks of cytoplasmic material in tissue culture (31).

The complex ultrastructure of cilia at the surface of cells has been recently described by Fawcett and Porter (32). Important and obvious questions are: How much destruction of cilia may be caused by respiratory viruses, and what sort of changes may be produced in these types of cells without actual cell destruction? The very presence of cilia on these cells emphasizes localization of cellular function and thus justifies our interest in cell surfaces in relation to virus growth.

Since we are emphasizing mechanisms of virus release from infected cells, we should remember that a number of studies on so-called apocrine gland cells, particularly those involved in the process of milk secretion, have shown that large secretory particles may be pushed out into the lumen of the glands and that with this a disintegration of the cell membrane may occur (fig. 11; refs. 33, 34). Cell particles other than the lipid and protein components of the milk should be found freely in the lumen. Thus, we may suppose that virus escapes from such cells without producing detectable pathological changes. We have summarized some ideas of virus release in the accompanying diagram (fig. 13).

It should be emphasized that although there is agreement concerning the limitation of the effect of some of these viruses to the surface of the cell, this is not true of all strains of these same viruses. A virulent strain of Newcastle virus destroys the cells and progresses through the allantoic cells into the blood vessels. The microvilli are often intact on top of a disintegrating cell. The mitochondria of the leucocytes and of the endothelial cells of blood vessels are swollen and those cells also disintegrate (26). A morphological representation of the difference between a virulent and an avirulent strain emphasizes the capacity of the virulent virus to destroy the cell and spread through the tissue by direct progression from cell to cell. This results in penetration into the capillaries and a subsequent viremia. The avirulent strain fails to destroy the cell and escapes only at the free surface into the allantoic sac (fig. 14). Thus, one would expect that both virulent and avirulent strains would attain similar high titers within a fluid medium such as the allantoic sac but that the virulent strain would be found in high titer throughout the embryo. This was found to be so (35).

This hypothesis of surface changes was tested in tissue cultures, and it was shown that both the avirulent and virulent strain will destroy cells when a large surface is presented to the virus; but, as expected, the virulent strain is much more effective and kills the cells quickly. The avirulent is slow and does not usually destroy macrophages (36).

The pox viruses, including fowl pox, vaccinia, and several others, grow in the cytoplasm and are sometimes contained within lipid inclusions. This was most strikingly demonstrated by Goodpasture in the case of fowl pox years ago (37). In the case of vaccinia, Bland and Robinow's evidence (8) that the inclusions of virus actually consist of colonies of virus particles has been confirmed. The fine structure of such virus particles in the cells themselves has been beautifully illustrated by Morgan (38). Changes in the mitochondria and an association of some of these virus particles with mitochondria (39) has not been apparent in the later studies (38, 40). However, no series of infected cells followed over a period of time has been reported, and this point remains unsettled. We may summarize by saying that this group of viruses grows readily in the cytoplasm and that their major effect is limited to that area.

Abnormal mitochondria as seen in the electron microscope have been found in cells infected with mammary tumor inciter, in some cases of mouse hepatitis and in chick embryos with herpes (figs. 12, 15, 16). This finding emphasizes the difficulty

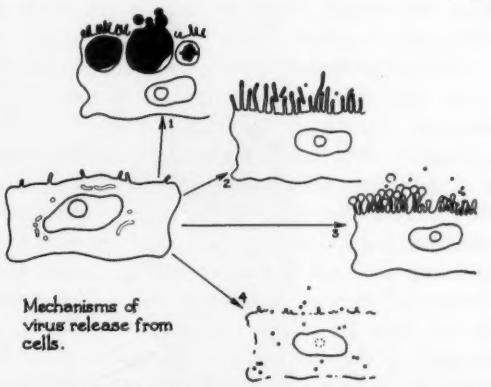

FIG. 13. Mechanisms of virus release from cells.

FIG. 12. Virus particles appearing in spontaneous mammary tumor cell, surrounding vesicles which are believed to be

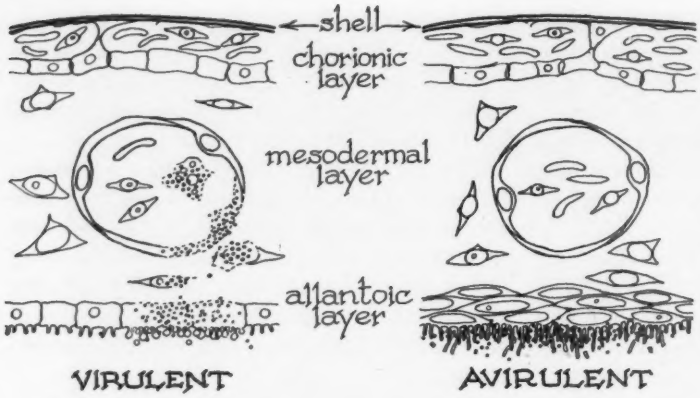

FIG. 14. Differential penetration of virulent and avirulent Newcastle.

of interpretation of an abnormal localization of enzymes within the mitochondrial fraction of tumor cells. The morphological changes in the mitochondria might be reflected in leakage of enzymatic activity either in or out of the mitochondria.

The specificity of these lesions in the mitochondria is by no means clear. Swelling and disintegration of mitochondria were described many years before the electron microscope existed. Now, higher resolution affords more detailed observation. With the electron microscope Rhodin has shown that the mitochondria of the kidney change markedly when albumen is excreted (41), and Weiss has shown that mitochondria close their folds and develop vacuoles and form the nidus for intracellular inclusions after neutral red is given (42).

Eastern equine encephalomyelitis, a small but destructive virus, has been followed primarily in tissue culture. It may be found apparently growing both at the edge of the cell and within the cytoplasm itself (figs. 17 and 18). Whether it derives sustenance from the ergastoplasm has not been determined. However, no selective effect of this virus on mitochondria has been observed.

Knowledge concerning the structure of the nucleus itself has not been greatly increased by electron microscopy. Although the nucleolus has a complex structure in many cells, it appears as a structureless diffuse mass. An increase in size of the nucleolus under a variety of conditions, including some virus infections, has been observed in living cells, and in the case of fowl plague, the 'inclusion' contains many particles similar in size to the virus. In fowl pox, the normal amorphous nucleoli of the chick chorio-allantoic membrane epithelial cells are changed into peculiar coiled structures (43). There is considerable structural similarity between this change and that described as unfolding of the nucleolus, which is brought about by the treatment of tissue culture cells with adenosine (44, 45).

The identification of virus particles, in particular in relation to the nucleus, is not without hazard. The finding by Gall of great numbers of small, uniform particles on the surface of the nuclei of oocytes of amphibians (46) should warn us that much of what is normal is still not known.

The study of herpes virus in cells has offered one of the greatest difficulties. T. McNair Scott and co-workers have for several years pointed out that changes within the nucleus may be seen early in the infection of the cells with this virus and they believed that the evidence of the accumulation of DNA within the infected nucleus was evidence of intranuclear growth of virus (47). The typical eosinophilic inclusions developed later. Early attempts at electron microscopy failed to show the virus particles within the nucleus (39) but recent studies of Morgan *et al.* (48) and of DeReissig and Melnick (49) have shown particles which are characteristic of virus in appearance. These are clearly within the nucleus and are present in clumps concentrated along the inside of the nuclear membrane. Further evidence of the intranuclear localization of the virus has been presented by Scott (50) who showed that when cells are fractionated early in the infection there is a much greater concentration of virus in the nucleus than when the fractionation is done later on disintegrating cells. The types of changes produced in tissue culture cells have been summarized in figure 19.

The most striking example of intranuclear localization is that of human warts. Bunting showed several years ago that masses of virus-like particles could be obtained from some of

FIG. 15. Liver cell infected with Nelson's hepatitis virus. Distended and ballooning mitochondria present in infected cell.
FIG. 16. Mesenchymal cell in chick chorioallantoic membrane infected with herpes. Distended vesicles with dense edges believed to be swollen mitochondria.

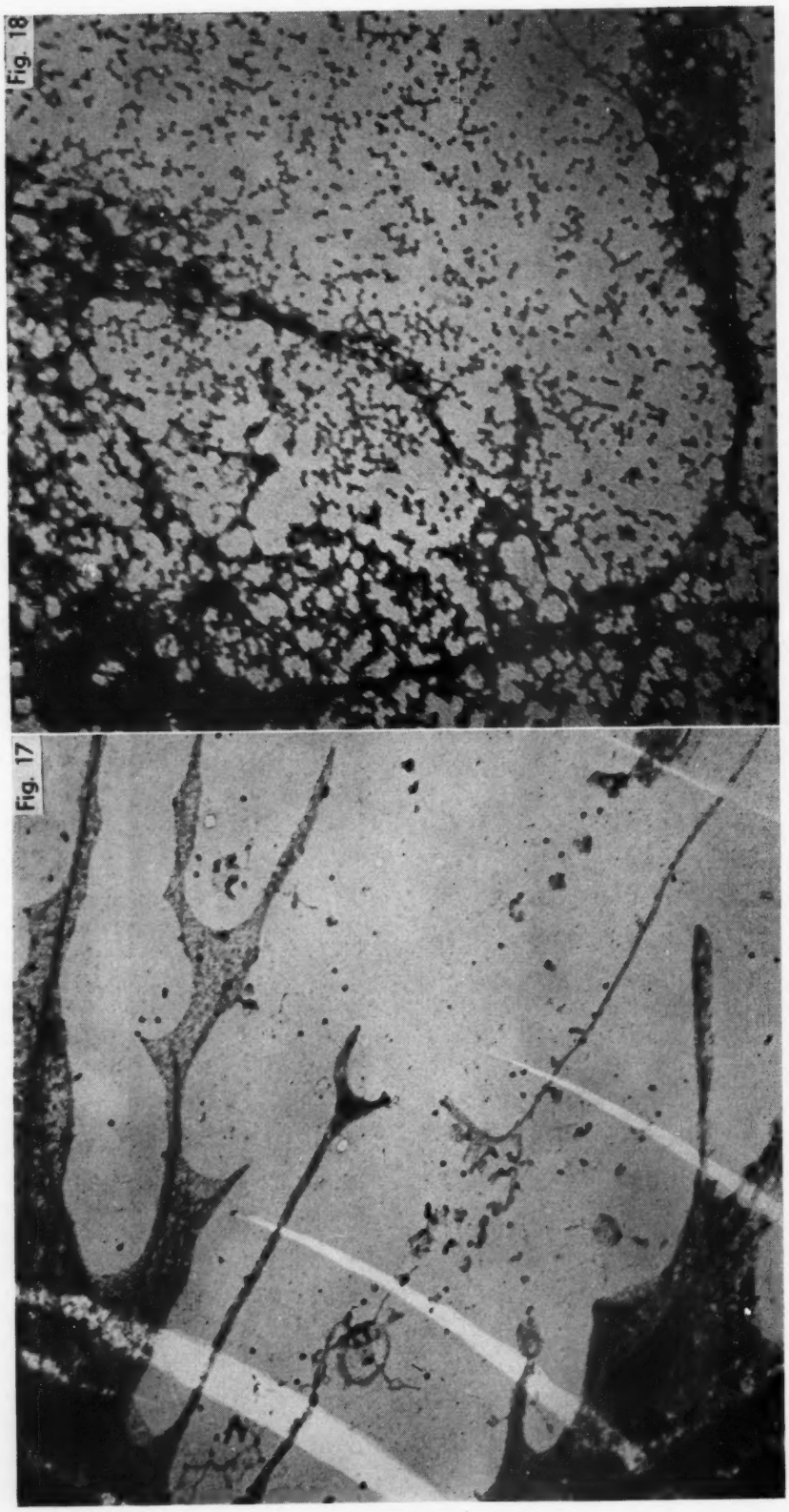

FIG. 17. Edges of rat fibroblast showing beginning destruction by Eastern equine encephalitis virus.
FIG. 18. Massive destruction of chick embryo fibroblast by Eastern equine encephalitis.

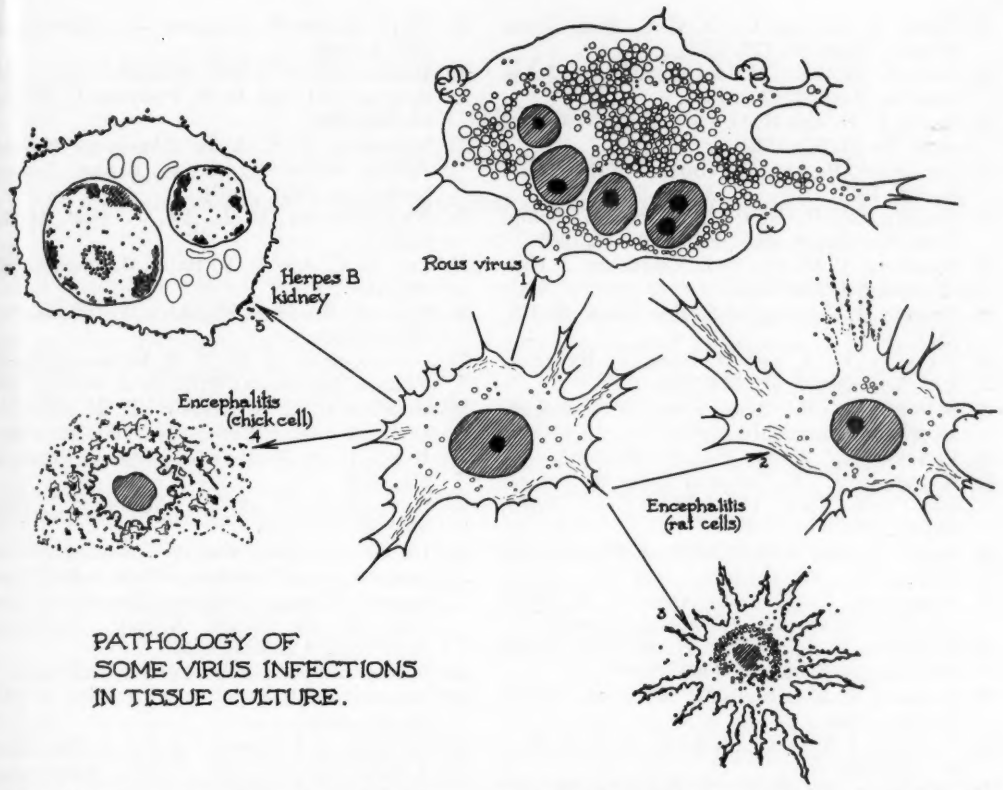

FIG. 19. Types of changes produced in tissue culture by different viruses. *1*, Rous virus—based on unpublished data of Lo, Sapras, and Gey. *2* and *3*, Encephalitis—based on data of Bang and Gey (9). *4*, Diagram of figure 18. *5*, Based on data of De Reissig and Melnick (49).

these by centrifugation. Electron microscope examination of sections of these warts has shown that almost the entire nucleus is replaced by virus particles (51).

Thus, it seems likely that some viruses grow readily within the nucleus, but we cannot yet tell whether the primary localization is intranuclear or whether the viruses grow readily in both cytoplasm and nucleus. The newer techniques, by which cells may be studied both in the living state and at ultra-resolutions, are beginning to give us new information concerning the relation of the virus and the cell. However, no matter how clear the effect of virus may be, virus itself is seen only if it is present in the cell in large amounts. In some of the problems of cellular pathology such as cancer the effect of a particular virus which is known to cause a particular tumor may occur in the absence of large amounts of virus. Indeed, a balanced condition between the virus and the tumor cell would almost presuppose a limited number of virus particles.

In summary, the pathologist who examines the cell can today speak of lesions of the surface of the cell, of destruction of pseudopods, of changes in the mitochondria, of virus within the nucleus, even of changes in the nucleolus. In relation to virus infection these changes are definite and clear, but the degree of specificity remains to be determined, and is an ever present challenge.

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FIG. 18. Massive destruction of chick embryo fibroblast by Lansing [illegible]

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RELATION BETWEEN CELL STRUCTURE AND CELL CHEMISTRY

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FOR A NUMBER of years in the past, many biochemists and cytologists had only one thing in common: they were both dealing with cells. The biochemist was dedicated, for example, to the purification of enzymes and the elucidation of the complex series of chemical reactions taking place in cellular metabolism, while on the contrary, the cytologist confined his interests to the morphological aspects of intracellular structures. Except for some hypothetical considerations, the obvious crux of the whole matter, i.e., the metabolic function of individual cell structures, was too often ignored, for at least one rather sad reason: the biochemist had inadequate training in cytology and the cytologist inadequate training in biochemistry. As a result, neither science was given serious consideration by the proponent of the other and there was a notable lack of collaborative effort. A somewhat analogous situation has occurred in every other branch of experimental biology, whether it be physiology, pharmacology, nutrition, or pathology; thus in almost all instances the fact cannot be escaped that the dynamic aspects of the activity of intracellular structures are being recorded and that further information is needed on the role played by these structures.

With the development of cytochemical methods (1–3), however, a beginning has been made toward the study of the relation between cell structure and cell chemistry. In terms of methodology, cytochemistry is still in its infancy; within the last 2 years, moreover, it has entered a new and difficult phase. Whereas the limited resolving power of the light microscope has for some time allowed the cytochemist a great deal of latitude in the interpretation of his data, the more recent improvements in techniques of electron microscopy (4–7), so ably demonstrated by Dr. Porter, have now pinned the cytochemist down. As you have seen, there is within the cell an array of complicated, highly organized structures that cannot be clearly resolved by light microscopy. If you are willing to agree that the method at present most capable of producing extensive cytochemical data is the isolation of intracellular structures by means of the centrifugal fractionation of broken cell suspensions (1, 2), then it must be obvious to you that the cytochemist is faced with some new problems. Since the sedimentation rate of a particle depends not only upon its size and shape but also upon its density, we may justifiably be asked, for example, whether isolated and presumably homogeneous preparations of microscopically visible structures (e.g., cell nuclei and mitochondria) are not extensively contaminated by particles of smaller size and greater density. We may also be asked what, from a cytological standpoint, is the composition of the so-called microsomal and soluble fractions of the cell. In answer to these questions, we can only say that the first poses a problem that may require some reinvestigation, and the second raises a point on which most of us have, fortunately, been non-committal (8, 9).

I might say in passing that although the electron microscopist has placed us in a difficult position, he himself is not exactly lying in a bed of roses. Thus, he too is dependent essentially upon a single technique of experimentation and is frequently faced with troublesome problems of interpretation. It is probable, however, that many of the present deficiencies and disagreements in electron microscopy will soon be resolved. During this period of rapid expansion in the knowledge of the fine structure of the cell (10–14), we, as cytochemists, must attempt to keep pace with the new cytological developments through a continued correlation of our chemical data with the latest morphological observations. In some instances, it is perhaps a little too early to make this attempt. The least that we can do now, however, is to appraise critically our present results, redefine our problems in the light of current cytological data, and try to find new and more definitive routes of experimentation.

It is therefore evident that the cytochemist must follow the lead of the cytologist, and must realize that data pertaining to the biochemical properties of a preparation of isolated cellular

particles have little cytochemical significance unless two important questions can be answered. First, what do the particles represent in terms of the organized structure of the cell and, second, what is the state of integrity and purity of the particles after isolation?

Since it is customary in summaries of this type to discuss each cell structure individually, the most obvious place to begin is with the nucleus. A number of methods, involving both aqueous solutions and organic solvents, have been devised for the isolation of cell nuclei (15–17). As far as we are aware, however, only one of these techniques has included quantitative cytological evidence for the purity of the preparations (15). In all other instances, the tests for homogeneity have been based mainly on cursory examination of the preparations in the optical microscope, a procedure that leaves much to be desired from the standpoint of objectivity and quantitation. In attempting to assess the data obtained from investigations of isolated nuclei, it is immediately evident that the role of the nucleus in cellular metabolism is obscured by lack of information concerning the permeability of the nuclear membrane. It has been claimed by Dounce *et al.* (16), Allfrey and his collaborators (17), and others (18) that the nuclear membrane is a relatively permeable structure, permitting in aqueous media the escape of water-soluble compounds, including proteins and substances of lower molecular weight. In order to avoid this difficulty, a procedure patterned after that originally described by Behrens (19) has been used for the isolation of cell nuclei. The tissue is dessicated in the frozen state, suspended in organic solvents of low polarity, and the nuclei isolated by centrifugation. Although this technique has a number of defects with respect to yield, determination of homogeneity, morphological integrity, and preservation of enzymes and other proteins in their native state, it should, at least in theory, prevent the extraction of water-soluble compounds. Unfortunately, however, the procedure has not yielded data indicating exclusive localization of anything other than desoxyribose nucleic acid (DNA) in the nucleus. These findings have a bearing on the philosophy under which one operates in cytochemical experimentation. It is our belief that the mere existence of specific intracellular structural entities is a reflection of a specific biochemical organization, and we would therefore expect to find that many compounds of biochemical importance are sharply localized within the cell. Although this concept has been amply justified by studies of isolated mitochondria, only two substances appear to be localized exclusively in isolated nuclei, namely, DNA and the enzyme that synthesizes diphosphopyridine nucleotide (20). The latter enzyme may, in fact, be nucleolar in origin (21). A number of other enzymes have been discovered in varying concentrations in isolated nuclei (22, 23), but the significance of these investigations is difficult to interpret in the absence of concrete evidence of homogeneity of the preparations. As a matter of fact, in an instance where the homogeneity could be objectively established, it was demonstrated that two enzyme systems previously found in isolated nuclei were actually localized in other cellular particles contaminating the preparations (15).

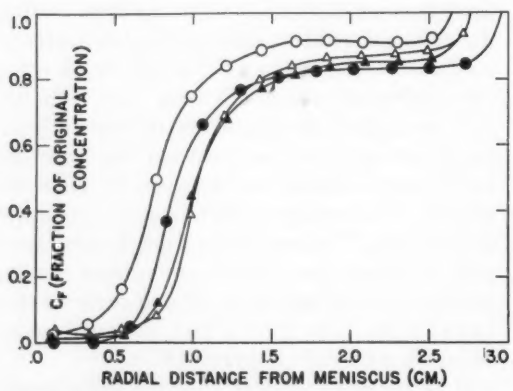

FIG. 1. Sedimentation diagrams obtained with monodisperse materials of widely different size ranges. For experimental details see text and ref. No. 44.

It should be mentioned, however, that despite observations with the light microscope indicating that the nuclear membrane is a solid, continuous structure, there is some evidence from electron microscopy that it may be interrupted here and there by a series of minute pores (24, 25). In an electron micrograph of a thin section of mouse liver prepared by Dr. A. J. Dalton (25), several pores in the nuclear membrane are clearly visible and at one site the interruption in the membrane appears to take the form of two small ergastoplasmic vacuoles. The implications of this observation are certainly far-reaching. Thus, it is possible that intranuclear material may be in direct contact with the cytoplasm and that the so-called nuclear membrane may be derived from the ground substance of the cytoplasm. This, of course, leaves us in somewhat of a dilemma, in that it could lead to the conclusion that

method for the isolation of nuclei is capable of yielding a true picture of their chemical constitution. Nevertheless, it should be remembered that electron microscopy has its vagaries and that there is evidence for the existence of a continuous nuclear membrane from other investigations. Furthermore, at least one water-soluble enzyme (the DPN-synthesizing enzyme) is sharply localized in isolated nuclei (20), and there is indirect evidence that the nucleus plays a prominent role in other synthetic reactions, notably that of ribose nucleic acid (26, 27). One is led to wonder, therefore, whether a more coherent knowledge of the biochemistry of the cell nucleus remains to be discovered. At any rate, perhaps it would be better for all concerned if we turned to the cytoplasm.

The most prominent component of the cytoplasm, at least as far as light microscopy is concerned, is, of course, the mitochondrion. Both cytochemical (8) and electron microscopical observations (4, 5) have established the specificity of this cytoplasmic structure. There is biochemical evidence that the double mitochondrial membrane demonstrated by Dr. Porter is relatively impermeable, remains so after cell disruption, and in many instances actually forms a barrier between substrate and enzyme (28, 29). We shall not dwell at length on a reiteration of the biochemistry of mitochondria, despite the fact that this information is derived from the soundest cytochemical data that are now available. The subject has, within the last few years, in fact, been reviewed almost to death (8, 9, 30–32). Suffice it to say that there is sound experimental evidence indicating that the mitochondrion is the center of the respiratory activity of the cell, in its content of cytochrome oxidase and most of the enzymes within and related to the Krebs tricarboxylic acid cycle. Other experiments (33–35) have shown that at least 50 per cent of the mitochondrial protein is present in the soluble state, the remainder being structurally bound, perhaps to the internal mitochondrial membranes seen in electron micrographs of thin sections.

As you have seen, the rest of the cytoplasm consists of a series of vacuoles, granules, and membranes that have been recognized only recently through the newer techniques of electron microscopy. There is, in fact, a considerable amount of debate as to the exact nature of these structures. Many of the smaller granules, including those first described by Palade (36, 14) and later by Sjöstrand and Hanzon (37), are of a size range corresponding to that of protein molecules and therefore may actually be in solution. In addition to these structures there is, in liver, a rather diffusely localized system of vacuoles, small granules, and double membranes that are extremely dense to the electron beam, particularly if fixation with buffered osmium tetroxide is followed by impregnation with unbuffered osmium tetroxide (38). This reaction is characteristic of the staining properties of the Golgi apparatus in other tissues where that structure is better defined (39), and the question arises as to whether the liver cell possesses a Golgi apparatus according to the classical definition.

If one assumes that the smaller cytoplasmic structures seen in electron micrographs do not vary greatly in their specific gravity, it is probable that most of them will appear in the microsomal fraction obtained by high speed centrifugation of tissue homogenates. That this fraction can be markedly heterogeneous, both chemically and structurally, is thus evident (8) and has been indicated by work in other laboratories (40–43). Furthermore, it should be noted that the size range of some cytoplasmic vacuoles overlaps that of small mitochondria. This fact suggests that the mitochondrial fraction itself is not as homo-

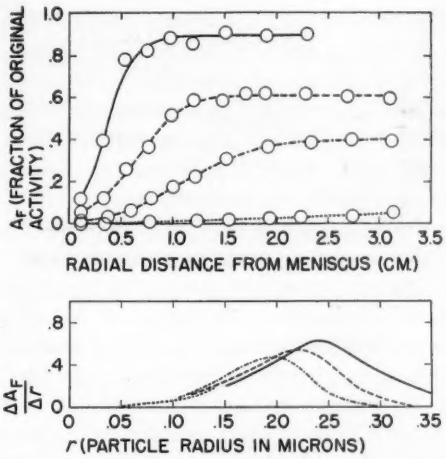

FIG. 2. *Upper curves:* Sedimentation diagrams of the succinic dehydrogenase activity of rat liver cytoplasm. From top to bottom, the rotational speeds were 3148, 4790, 6158, and 14,290 rpm. The time of centrifugation was 45 min. for the 6158 rpm run and 35 min. for the others. The average temperature was 4°. *Lower curves:* Concentration increment curves, translated from the sedimentation boundaries and showing the range of particle sizes observed (assuming a particle density of 1.2). For experimental details, see text and ref. 46.

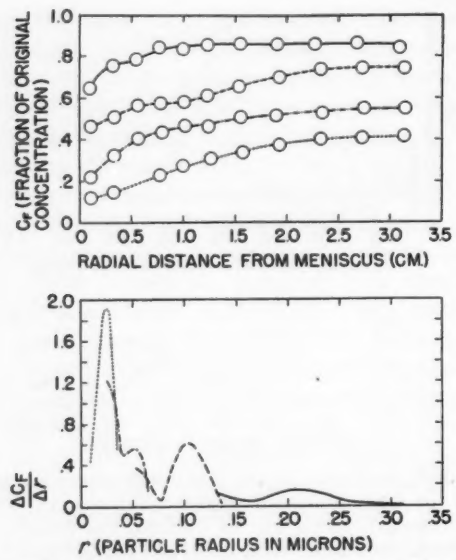

FIG. 3. *Upper curves:* Sedimentation boundaries of the ribose nucleic acid of rat liver cytoplasm. The rotational speeds varied between 4790 rpm for 35 min. to 39,460 rpm for 65 min. and the average temperature was 4°. *Lower curve:* A composite concentration increment curve, translated from the sedimentation boundaries and showing the range of particle size observed (assuming a particle density of 1.2). For experimental details, see text and ref. 46.

geneous as we previously supposed and could offer an explanation for the finding that some enzymes are not sharply localized in preparations of isolated mitochondria but are distributed, for example, between mitochondria and microsomes (8).

In order to carry out a biochemical study of the components of the cytoplasm, we have utilized a new technique of centrifugation, suitable for the attainment of high centrifugal forces and providing for a minimum of convectional disturbance (44). Homogenates of liver are first freed of nuclei and then centrifuged in a cylindrical tube in a horizontal, swinging-bucket type of preparative rotor (Spinco, Model SW-39). The fluid column is sampled at successive levels from top to bottom, and biochemical analyses are carried out on the fractions obtained. A sedimentation curve is constructed from the analytical data.

Several remarks in regard to the technique are in order. A slight density gradient within the fluid column is required to prevent undue convection during deceleration of the centrifuge. For this, we have used a medium consisting of hypertonic sucrose ranging linearly in concentration from 31% at the bottom of the fluid column to 27% at the top. Although we are by no means satisfied with the medium, hypertonic sucrose was chosen because it preserves the morphological integrity of mitochondria and does not induce aggregation of particulate material (2). One of the main difficulties in the procedure lies in the fact that the interpretation of sedimentation curves constructed from the sampling data requires extreme analytical precision. This, of course, is not a simple problem when one is dealing with the assay of enzymes (45).

In order to demonstrate the suitability of the method for particles of widely different size ranges, an initial study was made of the sedimentation behavior of a number of essentially homogeneous materials varying in size from that of the bovine albumin molecule, 60 Å in diameter, to that of the polystyrene latex particle, 2600 Å in diameter (44). In all instances, as shown in figure 1, reasonably sharp sedimentation boundaries were obtained. In this figure, the open circles represent bovine albumin, the closed circles polystyrene latex, the closed triangles rat liver catalase, and the open triangles lobster hemocyanin. The rotational speeds varied from 615 rpm for polystyrene latex to 39,460 rpm for the smaller proteins, and the initial concentration of sedimenting material ranged between 0.01 and 0.1%.

When the method was applied to the cytoplasmic particles of the liver cell, the boundaries as might be expected from morphological studies were much more diffuse. Figure 2, for example, shows the sedimentation boundaries obtained in several runs at different speeds, of succinic dehydrogenase, a property of mitochondria (46). The lower portion of the figure represents a translation of the boundaries into standard increment curves, indicating the range of particle size, assuming a particle density of 1.2 and a spherical shape. It is evident that the increment curves demonstrate the presence of a single family of particles with radii centering about 0.2 to 0.24 μ. The fact that all increment peaks do not coincide is probably due either to slight differences in the conditions of successive experiments or to variations in the size of mitochondria from animal to animal. In any event, the average observed radius correspond to the known dimensions of liver mitochondria. It may also be noted that the range of particle size is very wide, a few of the mitochondria having a radius of 0.1 μ or less

That this is not an artifact is indicated by the finding that extremely small filamentous mitochondria can actually be seen in the upper fractions after centrifugation of the fluid column. Small mitochondria can also be seen in fixed sections of liver, particularly in the non-parenchymal cells.

The picture given by the small cytoplasmic particles is much more complex. Figure 3 shows the distribution of ribose nucleic acid in a series of sampling experiments carried out at successively higher speeds, from 30 minutes at 4790 rpm to 1 hour at 39,460 rpm (46). It can be seen that the low speed run demonstrated a small but appreciable rise in increment in the region of the mitochondria. It may be noted that in the past, the small amount of ribose nucleic acid found in the mitochondrial fraction has been of doubtful significance because of the possible presence of other cellular particles (47). Three other increment peaks that are also present correspond to particles having approximate average radii of 0.025, 0.05, and 0.1 μ. The enzyme system that catalyzes the transfer of electrons between cytochrome *c* and diphosphopyridine nucleotide (DPN-cytochrome reductase) has much the same distribution as does ribose nucleic acid, except for the fact that the RNA-containing particles of an average radius of 0.1 μ are apparently inactive (46). Most of the acid phosphatase, on the other hand, is confined to the latter particles, the remainder of the activity being rather diffusely distributed (46). Uricase activity is entirely contained in the 0.1 μ radius particles (46).

In general, these data demonstrate that there are several distinct classes of cytoplasmic particles, each of which, under present conditions, is rather polydisperse in sedimentation characteristics. Furthermore, it is evident that the groups of particles overlap to an appreciable extent even when high resolution is obtainable through the use of a horizontal centrifuge. It is therefore obvious that previous methods for the fractionation of cellular particles in the angle centrifuge, which gives relatively poor resolution, leave much to be desired.

The techniques of centrifugation can thus be greatly refined, but we are nonetheless still operating in the dark from a morphological standpoint, particularly when dealing with particles smaller than mitochondria. What, then, do these particles represent in terms of cell structure? As a preliminary gesture in this direction, we have examined in the electron microscope two fractions, that containing the particles of 0.025 and 0.05 μ in radius and that containing the particles of 0.1 μ in radius. The fractions were fixed by the addition of neutral, buffered osmium tetroxide, dehydrated in ethanol, and imbedded in plastic. Thin sections were then cut and examined in collaboration with Dr. A. J. Dalton (48). We should hasten to say that the procedure employed was not entirely satisfactory, for at least two reasons. It was not possible to be certain that alterations did not take place during the procedure of fixation and imbedding, and difficulties were encountered in obtaining truly representative pictures of the fractions. The latter problem arose as a result of the fact that it was necessary to centrifuge the particles after fixation and during dehydration and imbedding. As might be expected, the fixed particles sedimented at a rate in accordance with their size and density and formed a pellet consisting of several distinct layers. At any rate, the larger particles, i.e., those of a radius centering about 0.1 μ (again assuming a density of 1.2) appeared to consist predominantly of ergastoplasmic membranes in the form of spherical vesicles, with many of the tiny granules described by Palade (39) still attached to the membranous structures. The slower sedimenting particles (those of a calculated radius of 0.025 and 0.05 μ) consisted mainly of vacuoles, some of which were larger, and therefore probably of a lower density than predicted. Although this fraction was cytologically inhomogeneous, it may be noted that many of the structures appeared to have double membranes and were rather dense to the electron beam. As we indicated earlier, these morphological characteristics suggest that the elements of the so-called Golgi apparatus were present. Nevertheless, in view of the heterogeneity of the preparation, it would be foolish of us to conclude definitively at this time that the particles that are rich in RNA and DPN-cytochrome reductase actually represent the Golgi substance, this despite the finding that the better defined Golgi apparatus of epididymus appears to contain a high concentration of RNA (49).

It is our hope that this summary will give you some idea of the current status of cytochemistry, at least insofar as cell fractionation experiments are concerned. As you can see, although the picture is at present not an altogether happy one, there is reason to believe that in the near future a knowledge of the relation between cell structure

and cell chemistry will be much more complete and will lead to an understanding of phenomena occurring in other areas of experimental biology. As far as our specific approach to cytochemical problems is concerned, several facts stand out. Recent techniques in centrifugation will certainly result in improved methods for the isolation of cellular structures, and recent advances in biochemistry will be of great aid in pointing toward metabolic reactions that warrant further study. We have the uncomfortable feeling, on the other hand, that the proper medium for the preservation of cell structures in the isolated state has yet to be discovered. Finally, it has become obvious that the cytological aspects of our problems require a great deal more emphasis, through joint efforts on the part of the biochemist and the cytologist.

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AMERICAN INSTITUTE OF NUTRITION

Symposium on Lipids in Nutrition[1]

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FAT AS A REQUIRED NUTRIENT OF THE DIET

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IT HAS LONG been recognized that fats and oils constitute foodstuffs possessing the highest caloric density. Until quite recently, they have been classified as optional components of the diet. As late as 1920, Osborne and Mendel (1) stated that "if true fats are essential for nutrition during growth, the minimum necessary must be exceedingly small." This opinion was expressed less than 10 years before the discovery by Evans and Burr (2) and prior to that of McAmis, Anderson, and Mendel (3), of the essential role of fat in the diet. These preliminary observations were followed by the classical studies of the Burrs, which resulted in the recognition of linoleic, linolenic, and arachidonic acids as the essential fatty acids (EFA).[2] The early work in this field was summarized in the first volume of FEDERATION PROCEEDINGS by G. O. Burr (4); the most recent summaries have been those of the present author and his collaborators, and of others (5–9). The failure of Osborne and Mendel (1) to recognize the necessity of fat for the growth of the rat may be due to the tenacity with which the EFA are retained in the tissues during subjection of the animals to fat-free regimens, and hence to the prolonged interval that is necessary before fat-deficiency symptoms can be demonstrated.

In the present discussion, the importance of fat in the diet will be reviewed, not only as reflected in the development of fat-deficiency symptoms, but also as related to the performance of such important physiologic functions as growth (which is considered along with the efficiency of food utilization and associative dynamic action), sex maturation, pregnancy, lactation, work capacity, length of survival during fasting (as related to fat in the previous diet) and nitrogen metabolism. Finally, it will be shown that the EFA are concerned with protection from x-irradiation injury, that the capillary permeability of the skin vessels is related to the ingestion of EFA, and that cholesterol deposition in the liver increases with dietary EFA deficiency. All of these data, relating the important physiologic functions to the dietary fat as a whole or to the proportion of EFA available, would seem to offer circumstantial evidence for the essential role which fats and oils play in intermediary metabolism.

PHYSIOLOGIC FUNCTIONS RELATED TO FATS AND OILS IN GENERAL

Growth. The rate and efficiency of growth have been the bases for evaluation in most studies on the nutritional value of foodstuffs. Although Osborne and Mendel (1) could not demonstrate on the basis of growth studies that fats are required in the diet, most recent tests have indicated a more intense rate of growth on fat diets as compared with that when fat-free regimens are employed; moreover, in a number of cases, progressively higher gains-in-weight have been noted in young rats when they were fed diets containing increasing proportions of fat. Tables 1 and 2 list the experimental data comparing growth on diets containing varying fat levels when ad libitum feeding and paired-feeding technics, respectively, were employed.

In most cases, the best growth has been obtained on diets containing 20–40% of fat by weight. In fact, the author (17) has suggested that the optimum level of fat in the diet as estimated from these tests would be approximately 30% by weight, or 50% on the basis of caloric

[1] San Francisco, April 13, 1955.

[2] EFA is used as an abbreviation for 'essential fatty acid(s).'

TABLE 1. COMPARATIVE GAIN-IN-WEIGHT OF YOUNG RATS FED DIETS CONTAINING SEVERAL LEVELS OF FAT WHEN DIETS WERE GIVEN AD LIBITUM

Fat Fed	Growth Response as Related to Fat Level	Ref.
Lard (4 types)	30% and 55% > 5%	(10)
Steam-rendered lard	30% > 15% and 54% > 5%	(11)
Cottonseed oil or margarine fat	20% > 40% > 14%, 10%, 5% > 0%	(12)

value. Scheer *et al.* (18) likewise observed that when weanling rats were stunted for 12 weeks by a drastic restriction in food intake, and the animals were then given diets ad libitum containing the several fat levels, markedly superior growth was noted in those receiving the 10%, 20%, and 40% fat diets as contrasted with those on the 0% or 5% fat levels. The increased potentiality in growth was particularly noted in the high-fat diets during the 9–12-week period following the restricted diets.

One might attribute the superior growth-promoting results obtained with the higher fat intake to a greater caloric consumption, since in many cases the diets were not isocaloric. Under such conditions, a higher caloric intake could be effected by the ingestion of the same amounts of high-fat diets as of low-fat regimens. However, this explanation does not account for the greater weight gains which have been observed where a paired-feeding technic was employed, and in which isocaloric diets were used (14, 19). One must look beyond the question of caloric intake for the explanation of the greater growth of rats on fat diets.

A better explanation for the superior effect of fat diets on growth is that this foodstuff exhibits a greater efficiency of utilization than do other foodstuffs. Swift and Black (20) reported that there was an increase in retained calories when a higher proportion of fat was fed, as a result of a reduced loss of calories as heat from the body. The pathways of the heat loss, when diets containing different proportions of fat were fed, are summarized in table 3.

The higher efficiency of fat calories appears to be the result of reduced heat loss on the higher fat diets. This phenomenon was discovered by Forbes and Swift (21), and was referred to as "associative dynamic action." Although Murlin and Lusk (22) had postulated that, in the case of the dog, the specific dynamic action of glycine, glucose, and fat was additive when the calorigenic agents were given together as well as when they were given

TABLE 2. COMPARATIVE GAIN-IN-WEIGHT OF YOUNG MALE RATS FED DIETS CONTAINING SEVERAL LEVELS OF FAT WHEN PAIRED-FEEDING WAS EMPLOYED

Fat Fed	Growth Response as Related to Fat Level	Ref.
Lard (Isocaloric diets)	30% > 10% > 5% > 2%	(13)
Lard	30% > 10% > 2%*	(14)
Lard	30% > 2%†	(15)
Lard	18%,≅ 11%,≅ 5%‡	(16)

* Greater gains at high fat levels associated with decreased heat production.

† Proportionally better results of high-fat diet on low protein (7%), as compared with high protein (22%).

‡ No statistically significant differences.

separately, Forbes and Swift (21) recorded opposite results for the rat. The discrepancy in results between these investigators may have been due to the fact that the tests were made on different species; however, it is more probable that it is due to the difference in technic employed, since the tests on dogs were carried out over a relatively short period after the foodstuff had been fed, while the rat tests were calculated on the basis of 24-hour respiration experiments. The greater capacity of fat, as compared with that of other foodstuffs, in producing growth in rats may well be due to the decreased heat production obtained with increasing proportions of fat in the diet which results from the reduced specific dynamic action (SDA)[3] under these conditions. The various SDA values observed in rats when comparable amounts of the several foodstuffs were fed separately or in combination have been presented diagrammatically by Forbes and Swift (fig. 1, ref. 21).

The greater efficiency of fat diets in producing growth and in reducing heat loss has been demonstrated for the natural triglyceride fat itself. Whether or not this property is one exclusively mediated by the proportion of EFA in the fat is not known. However, it is recognized that the extent of weight gain in rats receiving different levels of EFA is related to the quantity of the EFA available. This will be discussed later.

Sex Maturation. The period when maturity occurs is a fairly precise one; it can be determined with considerable accuracy in the case of the female from the time of opening of the vagina. Although this physiologic change has not ordinarily been employed as a measure of nutritional

[3] SDA is used as an abbreviation for 'specific dynamic action.'

TABLE 3. PARTITION OF ENERGY OUTPUT AND ENERGY RETAINED IN RATS RECEIVING ISOCALORIC DIETS CONTAINING DIFFERENT PROPORTIONS OF FAT (20)

Category	Diets Containing Fat Levels of 2%	5%	10%	30%
Energy intake, cal.				
Gross	2854	2854	2854	2854
Protein	799	799	799	799
Carbohydrate	1912	1713	1456	657
Fat	143	342	599	1398
Metabolizable calories	2601	2605	2610	2615
Energy output, cal.				
In feces	128	126	121	123
In urine	125	123	123	116
As heat	2195	2165	2154	2155
Energy retained, cal.	406	440	456	460

value, there is no reason why it should not be so used to evaluate the effectiveness of the diet. The data recorded in table 4 suggest that there is a marked decrease in the period required to attain maturity in the case of the female rat as the fat content of the diet is increased from 0 to 40%.

The results are quite decisive in demonstrating that maturation occurs earlier in female rats when they are given a fat-containing diet than when they are fed a fat-free one. This variation in maturition time has been shown to occur, not only in the case of females weaned at 21 days and given the diets ad libitum, but also in the case of young rats stunted for 12 weeks by an insufficient caloric intake and then continued on the same diets ad libitum.

It would seem from the results on the stunted group that fat per se is not the deciding factor in establishing maturation; more probably it is related to the increased rate of growth and development of the immature rat, made possible by the presence of fat in the diet. The maturation time is decreased to about the same extent in the case of normal rats fed diets containing 10%, 20%, and 40% of cottonseed oil as compared with the period required with diets containing 0% or even 5% of fat; in the second group with stunted rats, the best results were obtained on diets containing 10% of fat.

Pregnancy and Lactation. Pregnancy provides a more critical means of evaluation of the nutritional value of a diet than does growth, while the requirements for lactation are still more exacting. It has been repeatedly demonstrated that reproductive failure ensues in rats raised to maturity on fat-free diets (23–25). Quackenbush and coworkers (25) recorded that a single dose of 100 mg of ethyl linoleate given 3 weeks before the

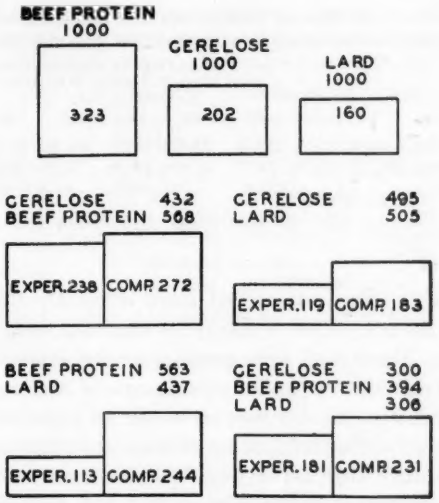

FIG. 1. Dynamic effects per 1000 calories of gross energy of nutrients as affected by nutrient combinations (21).

animals were bred was sufficient to ensure a normal pregnancy. Kummerow *et al.* (26) likewise attributed the beneficial effect of fat to the EFA, since hydrogenated coconut oil was ineffective at the 5% level, while corn oil was active. Deuel, Martin, and Alfin-Slater (27) also confirmed the necessity of EFA for successful pregnancy in rats, but the requirements found by these latter workers were somewhat higher than those recorded by Quackenbush *et al.* (25).

Although there are a number of negative reports in the literature as to the progressive improvement in pregnancy and lactation with increasing proportions of fat in the diet, Scheer and associates (18) did find an increase in the number of rats and in the total weights of the litters at 3 days and at 21 days as the proportion of fat was increased from 0% to 40%. These data are summarized in table 5.

The data in table 5 indicate that diets containing progressively higher contents of fat provide a better performance in some cases as regards pregnancy (3-day index) as well as lactation (21-day index). The role of the EFA in these physiologic functions is discussed later.

Work Capacity. The ability of tissues to continue to function over long periods obviously is a most important indication of the nutritional condition of the host. There does not appear to be any question that this capacity is a function of the quantity and type of food previously available to the animal. Krogh and Lindhard (28) reported

TABLE 4. EFFECT OF FAT LEVEL (COTTONSEED OIL) IN THE DIET ON TIME OF MATURITY OF FEMALE RATS

	Av. Time in Days to Maturity After Start of ad lib Feeding With Diets Containing Fat:				
	0%	5%	10%	20%	40%
Group I, 3 wk.*	60.2	49.0	45.5	44.8	44.1
Group II, 15 wk.†	14.7	11.9	10.5	7.7	9.8

* Fed ad lib. (12).

† Twelve wk. on restricted diet, then fed ad lib. (18).

that carbohydrate served more efficiently than did fat as a source of energy for muscular work in man. These data were contrary to the extensive and carefully controlled experiments of Anderson and Lusk (29) on dogs, in which an equal efficiency for the performance of muscular effort was indicated when fat or when carbohydrate served as the source of the energy. It is not generally believed that species variations exist in the efficiency of carbohydrate and fat as sources of energy. Because of the greater amount of data and the ability to establish more precise experimental conditions in the case of dogs than in human cases, it is the opinion of the author that the Anderson-Lusk data are the more authoritative.

More recent tests from two different laboratories have indicated that the capacity of rats to continue strenuous exercise is actually improved on high-fat diets as compared with that on regimens with a lower fat content or on fat-free diets. Thus, when swimming with progressively increasing loads was employed as the experimental stress (30), the duration of the swim was greater in practically all cases when the animals had been receiving a fat diet as contrasted with a fat-free diet (12). In some instances, the period of the swim was progressively increased when diets with higher proportions of fat had been consumed. These results are summarized in table 6.

Samuels, Gilmore, and Reinecke (31) also obtained a superior work performance in rats, when fat diets were employed, by the use of an entirely different technic. The experimental animals were fed diets containing 80% of fat, carbohydrate, or protein for 28 days previous to the test; the balance of the diet consisted of vitamins, salts, and minimum amounts of the other foodstuffs. The animals were subsequently fasted, during which period the amount of activity was recorded, together with the period of survival. Table 7 summarizes these results.

Although the amount of spontaneous activity of the rats previously fed fat does not differ

TABLE 5. REPRODUCTION AND LACTATION PERFORMANCE OF FEMALE RATS RECOVERING FROM A PERIOD OF UNDERNUTRITION AND RECEIVING DIETS CONTAINING COTTONSEED OIL AD LIBITUM (18)

Diets		Av. No. Rats/ Litter	Av. Wts. at 3 Days		Av. Wts. at 21 Days	
No.	Fat Cont.		Litter	Rat	Litter	Rat
	%	gm	gm	gm	gm	gm
606*	0	5.3	47.6	8.9	152	26.1
61	5	6.8	47.7	7.1	219	32.4
62	10	7.7	64.3	8.4	167	27.8
63	20	8.5	60.8	7.2	230	31.8
64	40	8.9	72.3	8.1	283	30.6

* Containing linoleic acid supplement.

appreciably from that of the group which has been prefed with carbohydrate, there is a marked superiority in both groups over the spontaneous activity of the protein-fed animals. On the other hand, the physical capacity of the fat-fed group, as determined from forced activity, is the highest of any of the three groups. This result can be attributed only partly to the increased survival period of the fat-fed group over the groups fed carbohydrate or protein.

Survival Time During Fasting. The period of survival of rats subjected to fasting is greatest when they have received a high-fat diet during the prefast period; the previous consumption of either fat or carbohydrate was found to afford more prolonged survival than did protein. These data are summarized in table 7.

Roberts, Samuels, and Reinecke (32) also noted that the survival of rats following evisceration was markedly prolonged when they had received the high-fat diet, as contrasted with the high carbohydrate diet, for 3–6 weeks before the surgery. The tests were carried out after a 30-hour fast. The average survival time was as follows: high-fat group, 17.0 hours (5 rats); high-carbohydrate group, 8.8 hours (6 rats).

The prolonged survival time when the animals are prefed with fat, as contrasted with prefeeding with carbohydrate, is to be ascribed to the establishment of a different metabolic pattern in the first instance. Thus, Roberts *et al.* (32) recorded an apparently slower rate of glucose utilization after evisceration in the previously fat-fed group and the absence of hypoglycemic symptoms preceding death, although the oxygen consumption was parallel in both dietary groups. It was shown in the case of intact fasted rats, that those previ-

TABLE 6. WORKING CAPACITY AS DETERMINED BY SWIMMING TESTS OF RATS WHICH HAD RECEIVED DIETS WITH DIFFERENT FAT LEVELS FOR 6 OR 12 WEEKS PREVIOUSLY (12)

Dietary Fat	Duration of Swim Using Rats on Diets With Fat Content:						
	0%	5%	10%	20%	30%	40%	50%
Male Rats							
Cottonseed oil	641 (39)	769 (19)	*850* (17)	846 (19)	—	*1068* (15)	—
Margarine fat	485 (23)	—	*806* (10)	*1174* (8)	*1065* (25)	876 (4)	*1122* (10)
Female Rats*							
Cottonseed oil	546 (10)	802 (6)	777 (5)	*1062* (5)	—	922 (3)	—

Figures in italics differ statistically from those obtained in the 0% fat group. Figures in parentheses are number of tests.

* Six wk. on diet only. Other groups on diet for 12 wk.

TABLE 7. EFFECT OF PREVIOUS DIET ON SPONTANEOUS AND FORCED ACTIVITY OF RATS DURING FASTING, AND ON PERIOD OF SURVIVAL (31)

	Fat*	Carbohydrate*	Protein*
	Spontaneous Activity, Cage Revolutions		
2–4 days	6730±915(12)	4040±411(16)	3600±262(7)
9–11 days	8800±1020(13)	8660±1017(14)	7220±500(4)
16–18 days	7960±738(7)	8650(3)	(0)
	Forced Activity, Cage Revolutions		
	19700±1780(12)	13800±1630(14)	6465±1650(9)
	Survival Time, days		
	18.3±0.44(12)	15.5±0.46(13)	10.2±1.68(9)

Figures in parentheses represent the number of rats in each group.

* Diet for 28 days prior to test.

usly receiving a high-fat ration had a markedly ower susceptibility to insulin than did animals which had received a high-carbohydrate regimen during the prefast period (33, 34). This effect was scribed to a prolonged retention of liver glycogen ue to the fact that the animals develop a sparing ction for this liver glycogen (32).

The variability in utilization of carbohydrate as elated to previous diet can be demonstrated at he level of isolated tissues. Thus, Gilmore and amuels (35) noted that tissues from animals reviously on the high-fat regimen utilized lucose much more slowly than did those previusly receiving carbohydrate. These results support the data of Lundbaek and Stevenson (36), who observed that the diaphragm of the carbohydrate-fed rat utilized carbohydrate at approximately twice the rate observed for the comparable issue from the fat-fed rat.

Nitrogen Metabolism. It was formerly considered that fat is entirely ineffective as a protein-parer. In the classical experiments of Graham usk (37), it was found that, on a constant protein ntake, carbohydrate, but not fat, was able to decrease the urinary nitrogen. However, in the last w years, it has been reported that certain hases of protein metabolism are influenced by fat ther than by carbohydrate.

One phase of protein metabolism responsive to t is the so-called 'wear and tear quota' or nitroen minimum. This value is the minimum level of inary nitrogen which is produced on a protein-free diet. Swanson and her collaborators (38, 39) observed that the nitrogen minimum can be produced in rats fed the full requirement of a protein-free diet, irrespective of whether or not fat is present in the diet. However, when the caloric intake was reduced to 50% of that required, the nitrogen minimum was maintained at a low value only if 20% of fat was included in the protein-free diet. Even when the caloric intake was reduced to 25% of that required, the urinary nitrogen was only slightly increased when fat was included in the diet, while it was very greatly augmented in the group receiving the fat-free regimen. According to Schwimmer and McGavack (40), fat exerts a similar sparing action on the level of the nitrogen minimum in man. Table 8 summarizes some of the data on rats.

When rats on protein-free regimens were supplemented with methionine, there was a smaller loss of urinary nitrogen when the diets were administered at 50% or at 25% of the caloric requirement than in the absence of this amino acid. However, here again the greater protein-sparing action obtained when fat was included in the diet.

A second instance in which the extent of protein catabolism is reduced by fat (but not by carbohydrate) has been reported by the Samuels group, who used the technic described earlier (31). During the period of fasting, following a 28-day period during which diets made up almost exclusively of protein, carbohydrate, or fat had been given to rats, the lowest excretion of urinary nitrogen was noted in the group receiving the fat diet. This may be one factor which enabled this group to survive longer. The nitrogen excretion per unit of work was likewise found to be the least in the previously fat-fed group. These data are summarized in table 9.

Samuels and collaborators (31) reported

TABLE 8. AVERAGE NITROGEN EXCRETION OF RATS FED PROTEIN-FREE DIETS WHICH WERE FAT-FREE OR CONTAINED VARIOUS PERCENTAGES OF REQUIRED CALORIC LEVEL (38)

% Caloric Requirement Fed	Av. Urinary N		Dev. From Control	
	Fat-free	20% Fat	Fat-free	20% Fat
	mg	*mg*	*mg*	*mg*
100	185	210	−95	−85
75	211	263	−61	−47
50	594	289	+311	+24
25	818	544	+569	+254

TABLE 9. AVERAGE NITROGEN EXCRETION (GM/RAT/DAY) OF RATS DURING A PERIOD OF FASTING, FOLLOWING A PERIOD DURING WHICH DIETS WERE COMPOSED ALMOST EXCLUSIVELY OF PROTEIN, CARBOHYDRATE OR FAT (31)

Fast, days	After Protein	After Carbohydrate	After Fat
Before	1.369±0.38	0.338±0.005	0.316±0.00
0–4	0.255±0.003	0.177±0.007	0.110±0.00
6–11	0.143±0.04	0.132±0.011	0.100±0.00
13–end		0.229±0.013	0.141±0.02

that, when the fasting rats were exercised, the nitrogen excretion per unit of work was as follows, according to the prefast diet: protein, 0.052 ± 0.01 gm/day/rat; carbohydrate, 0.046 ± 0.006 gm; and fat, 0.034 ± 0.004. Thus, it would appear that the conditioning of rats to high-fat diets allows them subsequently to carry out work with less destruction of protein stores than when the conditioning has occurred with other foodstuffs.

Other types of experiments have also demonstrated that fat may exert a superior action in sparing protein. Thus, French, Black, and Swift (15) reported a more rapid gain-in-weight and a more efficient use of the foods by rats on a low-protein diet when the basal diet contained 30% of fat than when only 3% of this foodstuff was present. Salmon (41) and later Geiger (42), noted that cystine spares protein only when fat is included in the diet.

As another instance of the superior protein-sparing action exerted by fat, one may cite the results of Rogers *et al.* (43). These investigators reported that the nitrogen balance was more favorable in protein-depleted rats from which 70% of the liver had been removed, when 30% of fat was included in the diet, than when only 3% of this foodstuff was fed. These beneficial results of the fat diets on protein retention occurred when paired-feeding based upon caloric consumption was employed.

PHYSIOLOGIC FUNCTIONS RELATED TO ESSENTIAL FATTY ACIDS

In addition to the experimental evidence for the essential nature of fat which can be deduced from the comparative effects of diets containing or free from this foodstuff, respectively, on growth, sex maturation, pregnancy, lactation, work capacity, length of survival, and nitrogen metabolism, the data obtained by tests with EFA provide strong support for this viewpoint. However, until recently the chief indication of the necessity of the EFA has been the proof that deficiency symptoms develop when they are absent from the diet. The chief deficiency symptoms which have been recognized have been a scaly skin and caudal necrosis, a marked retardation in growth, kidney lesions with a concomitant hematuria, increased water consumption, and death. The fact that the deficiency symptoms can be produced in a wide variety of animals confirms the concept that the nutritional importance of the EFA represents a general phenomenon. Although Gröer (44) and Hansen (45) presented experimental evidence for the requirement of EFA by infants to prevent or to cure a characteristic type of eczema, there are no corresponding data which indicate an EFA requirement in the adult human. However, Lambert *et al.* (46) have recently demonstrated that characteristic deficiency symptoms develop in the calf in the absence of EFA; the nutrition of this species has long been considered to be independent of the EFA.

Growth. The relationship of EFA intake to growth has recently been more completely investigated. Thus, Greenberg *et al.* (47, 48) demonstrated that the extent of increase in weight of young male rats whose body weight had reached a constant value due to EFA deficiency was directly proportional to the log dose of linoleate fed when the daily intake was between 5 and 100 mg per rat per day. Later experiments (49) indicate that this proportionality may likewise obtain at dosages as high as 200 mg per day. On the other hand, the maximum value at which a corresponding straight-line function between weight gain and log dose has been noted in young female rats is 50 mg per rat per day, and it may be even lower than this value (47).

Pregnancy and Lactation. Although the neces-

TABLE 10. REQUIREMENT OF EFA (FED AS COTTONSEED OIL) DURING PREGNANCY FOR NORMAL REPRODUCTION OF YOUNG FEMALE RATS PREVIOUSLY ON A FAT-FREE DIET, AS REFLECTED BY PUPS BORN AND BY SURVIVAL FOR 3 DAYS (27)

	Daily Cottonseed Oil Fed to Mothers, mg						
	0	10	40	100	200	400	1000
Females bred	23	24	25	25	25	25	25
Litters cast, %	83	96	92	100	96	92	100
Litter (at birth)							
Total no. rats	122	196	192	228	210	227	232
Av. no./litter	6.4	8.5	8.3	9.1	8.8	9.9	9.3
Mortality (0–3 days), %	100	76	39	29	23	14	25
Litter (at 3 days)							
Total no. rats	0	46	117	162	162	196	175
Av. wt./rat, gm		4.7	6.1	6.3	6.8	6.4	6.6

TABLE 11. REQUIREMENT OF EFA (FED AS ETHYL LINOLEATE) DURING PREGNANCY FOR NORMAL REPRODUCTION OF YOUNG FEMALE RATS PREVIOUSLY ON A FAT-FREE DIET, AS REFLECTED BY PUPS BORN AND BY SURVIVAL FOR 3 DAYS (27)

	Daily Linoleate Fed to Mothers, mg						
	0	2.5	5.0	10	20	40	80
Females bred	16	15	16	16	16	16	16
Litters cast, %	87	93	87	100	100	94	100
Litters (at birth)							
Total no. rats	69	93	96	111	120	108	115
Av. no./litter	4.9	6.6	6.8	6.9	7.5	7.2	7.2
Mortality (0–3 days), %	100	100	100	87	43	27	14
Litter (at 3 days)							
Total no. rats	0	0	0	14	68	79	99
Av. wt/rat, gm				5.0	4.9	5.6	6.5

sity of the EFA for reproduction was among the first of their functions to be recognized (23–25), quantitative aspects of such requirements have only recently been investigated. According to Deuel *et al.* (27), fat is not required by the female rat for conception or for the completion of pregnancy; however, the pups are invariably born dead or die immediately after birth when EFA are absent from the mother's diet. Daily doses of cottonseed oil as low as 10 mg allow some survival of the young for as long as 3 days; as much as 200 mg (containing approximately 100 mg linoleate) per day are required for optimum survival. Comparable effects can be produced with daily doses of 80 mg of ethyl linoleate. These requirements for EFA are considerably higher than those recorded by Quackenbush *et al.* (25).

The requirement for EFA remains high during the lactation period. Thus, no young survived until weaning (21 days) when the mothers received 10 mg of linoleate daily. The lowest mortality rates, and the highest weaning weight, of the pups at 21 days (which approached that observed on good natural diets) were noted in the groups in which the mothers received 100 or 200 mg of cottonseed oil daily (approximately 50 or 100 mg linoleate), or 80 mg of linoleate as such, during the lactation period. These data are summarized in tables 10–13.

Protection Against X-Irradiation. The important role which the EFA play in the metabolic processes of the body is indicated by the fact that they have a protective effect against the toxic symptoms produced by x-irradiation injury. In 1950, Decker and co-workers (50) observed that a typical EFA deficiency could be enhanced in fat-deficient mice when they were subjected to x-irradiation. Conversely, Cheng and her associates (51) found that cottonseed oil exerts a protective effect against x-irradiation in the rat.

A number of circumstances have been shown to be involved in the action of fat under such conditions. In the first place, it varies with sex. The protective effect of cottonseed oil against death from x-irradiation was found to be readily demonstrable in the young mature male, but not in the young mature female (51). This phenomenon is apparently associated with a greater resistance exhibited by the female to x-rays, both as demonstrated following multiple sublethal doses (51) administered at varying time intervals (52) and also as observed from the LD_{50} determined with single doses of x-rays of varying intensity (53). It is suggested that the greater resistance of the young females to x-irradiation may be related to the fact that the females have a lower requirement for EFA than is the case with males. Therefore, the females cannot be as completely depleted of EFA, within a given period, as can the males. The result is a residual protection against x-irradiation injury due to the EFA still present in the tissues. When the protective effect of cottonseed oil against x-irradiation injury was studied in very old rats, it was found that both males and females are equally protected. It seems probable that this fact may be due to the disappearance of the sex difference in fat metabolism under such conditions; this cessation in sex difference has been demonstrated by the uniformity of liver glycogen values in

TABLE 12. REQUIREMENT OF EFA (AS COTTONSEED OIL) FOR LACTATION OF YOUNG FEMALE RATS PREVIOUSLY ON A FAT-FREE DIET AS REFLECTED BY BODY WEIGHT AND SURVIVAL OF PUPS AT WEANING (21 DAYS) (27)

	Daily Cottonseed Oil Fed to Mothers, mg						
	0	10	40	100	200	400	1000
Litter (21 days)							
Orig. no. of litters	19	23	23	25	24	23	25
Litters represented		3	16	21	18	20	21
No. rats	0	9	88	124	114	122	126
Av. wt/rat, gm		24.6	26.9	29.4	33.4	32.0	29.4
Mortality (3–21 days)*							
Total		32	9	11	18	22	14
%		77	9	8	14	15	11

* Litters cut to 7 at 3 days.

TABLE 13. REQUIREMENT OF EFA (FED AS ETHYL LINOLEATE) FOR LACTATION OF YOUNG FEMALE RATS PREVIOUSLY ON A FAT-FREE DIET, AS REFLECTED BY BODY WEIGHT AND SURVIVAL OF PUPS AT WEANING (21 DAYS) (27)

	Daily Linoleate Fed to Mothers, mg						
	0	2.5	5.0	10	20	40	80
Litter (21 days)							
Orig. no. of litters	14	14	14	16	16	15	16
Litters represented					8	10	13
No. rats	0	0	0		44	58	77
Av. wt/rat, gm					22.9	25.7	29.2
Mortality (3–21 days)*							
Total				13	21	11	12
%				100	32	16	13

* Litters cut to 7 at 3 days.

unfasted animals and by similar levels of exogenous ketonuria in old male and female rats, respectively. The comparative average length of survival of young and old rats, which had been x-irradiated, on fat-free and fat diets is shown in table 14.

In subsequent tests (54) it was proved that the protective effect of cottonseed oil against x-irradiation injury is a function of the EFA present in it. When 10-mg doses of ethyl linoleate were given to rats on a fat-free diet and the animals were exposed to weekly doses of 300 r of x-rays, a significantly greater survival rate was noted in the case of the supplemented animals than in the case of the control rats, in spite of the fact that the former group received, on an average, as much as 45% more x-irradiation than did the control group.

The optimum EFA requirement to protect from x-irradiation injury is an appreciable one. Thus, it was found (55) that the LD_{25}, LD_{50}, and LD_{75} were progressively higher when the daily dosage of linoleate given the rats on the fat-free diet was 0, 10, 50, and 100 mg. This is illustrated in table 15.

Relation to Capillary Fragility. Another physiologic function which is related to EFA is capillary permeability. Kramár and Levine (56) conclusively demonstrated that fat-deficient rats had a high capillary permeability (or lower capillary resistance) as determined by tests of the capillary resistance of the skin vessels, as well as by capillary permeability, as determined by the McClure and Aldrich test and by the intraderma dye-spread method. When rats on the basal die were given 5% of methyl stearate, no improvement was noted; however, when linseed oil o linoleic acid was given, capillary resistance wa restored immediately to its normal high value This behavior explains the observation of Kramá and Kovács (57), made 14 years earlier, that th capillary resistance in human subjects was highe in the case of patients who had received vegetabl oils than in those who had partaken exclusivel of animal fats (presumably those which are lo in EFA).

It is possible that the greater resistance t

TABLE 14 (DAYS). AVERAGE LENGTH OF SURVIVA OF YOUNG AND OLD MALE AND FEMALE RATS O FAT-FREE AND ON COTTONSEED OIL DIETS AFTE MULTIPLE SUBLETHAL DOSES OF X-RAYS (51)

	Av. Survival of Old Rats (18 mon.)		Av. Survival of Young Rats (3 mon.)	
	Males	Females	Males	Female
Fat-free diet	34.3±3.3	39±4.3	51±3.1	61±3.
Cottonseed oil (2%)			65±3.0	67±2.
			3.25	*1.3*
Cottonseed oil (15%)	70.6±6.1	76±7.0		
	5.22	*4.52*		
Cottonseed oil (30%)	63.7±7.1	69±5.3	67±3.2	62±3.
	3.61	*4.42*	*3.60*	*0.2*

Including Standard Error of Mean. Values italics represent ratios of Mean Difference Standard Errors of Mean Difference (M.D S.E.M.D.).

TABLE 15. SURVIVAL OF MALE RATS ON FAT-FREE DIET, WITH OR WITHOUT ETHYL LINOLEATE, SUBJECTED TO X-IRRADIATION OF 200 R WEEKLY FOR 10 WEEKS (55)

	Ethyl Linoleate Intake Daily, mg			
	0	10	50	100
Group No.	*I*	*II*	*III*	*IV*
Days reached,				
25%	28	54	71	86
50%	54	73		
100%	69			
Av. survival time, days*	50.7±5.8	71.5±5.7	83.8±3.4	87.4±2.0
S.D.:S.E.M.D.† compared with *Group I* (control)		2.70	4.93	5.98
Surviving rats at 91 days				
No.	0	5	11	9
%	0	33.3	73.3	69.2

* When this value exceeds 3.0, the results are considered to be highly significant.

† See footnote to table 15.

noxia, which has recently been observed in our laboratory in animals receiving a cottonseed oil diet, as compared with those on a fat-free one, is a reflection of the greater capillary resistance in the former case.

Relation to Cholesterol Metabolism. One of the most important functions of the EFA appears to be in the regulation of cholesterol metabolism. It has long been known that, in the case of the blood, the cholesterol esters are composed of the most highly unsaturated fatty acids. Thus, Kelsey and Longenecker (58) reported that 62% of the cholesterol in beef plasma was in combination with linoleate; this proportion of linoleate is many times higher than that in other fractions of the blood lipids.

Although the administration of a fat-free diet to rats has recently been found to lower the level of blood cholesterol, Alfin-Slater and co-workers (59) have recently reported that a concomitant increase of liver and adrenal cholesterol of a considerable magnitude occurs. Under these conditions, typical fatty livers having an increased fat content were observed. On the other hand, when a cottonseed oil diet was fed, the blood cholesterol was not reduced, and, furthermore, no simultaneous rise in liver or adrenal cholesterol levels was noted. The beneficial effect of the cottonseed oil is presumably due to the EFA, since a similar decrease in liver cholesterol can be effected with linoleate (60). The decrease in blood cholesterol, together with the augmentation of liver cholesterol when fat-free diets are fed, has been found to develop within 1 week from the initiation of a fat-free diet; the variations continued at an increased level over a 13-week period, during which the fat-free diet was given. These results are shown graphically in figures 2 and 3.

One must conclude that the EFA are required to bring about a normal distribution and metabolism of cholesterol. Possibly, this alcohol cannot readily be transferred to other tissues from the liver in the absence of EFA. This would account for the accumulation of cholesterol. The amount of cholesterol combined with linoleate in the liver is very small, irrespective of the proportion of EFA in the diet (61). When fat-free diets are given to rats, the saturated acids tend to replace oleic acid in the cholesterol esters in the liver, and no change occurs in the insignificant quantity of cholesterol linoleate present in this organ (61).

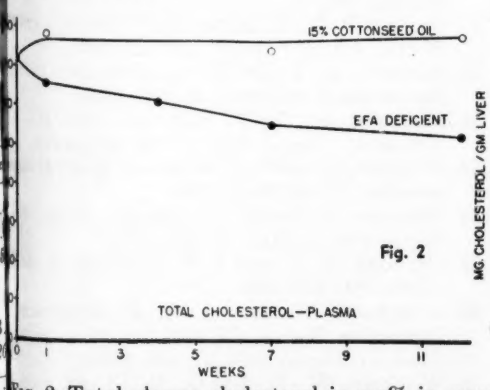

Fig. 2

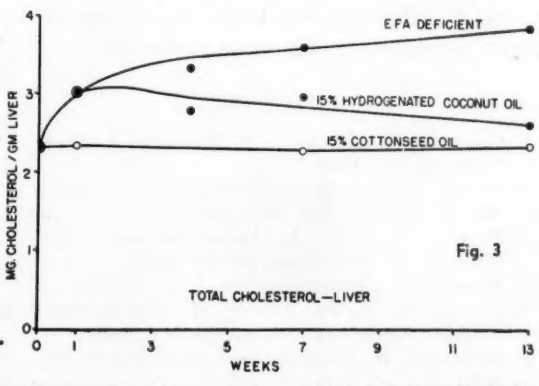

Fig. 3

FIG. 2. Total plasma cholesterol in mg% in weanling male rats receiving a diet deficient in EFA (*lower curve*) or one containing 15% refined cottonseed oil (*upper curve*) over a 12-week period (60).

FIG. 3. Total cholesterol in the livers of rats in mg/gm. Rats had received from weaning a diet deficient in EFA (*upper curve*), one deficient in EFA but containing hydrogenated coconut oil (*middle curve*) or one containing 15% refined cottonseed oil (*lower curve*) over 13 weeks (60).

CONCLUSION

It is as yet a moot question whether the beneficial effects of fats, nutritionwise, are to be ascribed solely to the EFA which they contain or whether triglyceride fats have a specific nutritional value per se. The beneficial effects of high-fat diets on pregnancy, lactation, and possibly also on growth can probably be largely, if not entirely, ascribed to their EFA content. On the other hand, there is no positive evidence that the improved caloric efficiency resulting from the associative dynamic effects, or the sparing action of fats, on certain phases of protein metabolism are necessarily functions of the EFA.

Some of the beneficial effects of fat are undoubtedly to be traced to the establishment of definite enzyme patterns when high-fat diets are consumed over an extended period. The longer survival on fasting has been shown to be related to a slower disappearance of glycogen than occurs in animals conditioned with high-carbohydrate diets. It may be that the increased capacity for work, noted in rats receiving high-fat diets, is a reflection of the establishment of such new enzyme patterns.

The explanation for the protective effect of the EFA against x-irradiation injury is not immediately forthcoming. The fact that the relative behavior of linoleate and that of linolenate are similar, not only as regards growth (47), but also in affording protection from x-irradiation injury (62) and in supporting pregnancy (63), would seem to indicate that a number of these phenomena are related. One is forced to the conclusion that, from a nutritional standpoint, fats have specific functions not shared by the other foodstuffs. They can no longer be considered simply as optional components of the diet.

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EVALUATION OF FAT-SOLUBLE VITAMINS

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IN THE TIME allowed for this topic one could not discuss fully even one essential fat-soluble nutrient. I shall instead attempt to present a few points concerning *a)* the relation of vitamin intake to measurable biological responses such as growth rate, calcification of bones, blood clotting, power, etc.; *b)* the comparative potencies of a vitamin and its precursors; and *c)* estimation of nutritional requirements.

In this discussion I shall apply to nutrition data the principle which I prefer to call the 'law of diminishing returns.' This principle is by no means new. It is known equally well to the economist and to the chemist, who calls it the first-order reaction rate law.

This principle was employed years ago by myself in the analysis of data on vitamin K requirement as related to blood clotting power (1). Hegsted (2) applied the principle in the study of the riboflavin requirement of ducklings, and gave a thorough discussion. I have found the principle useful in the study of data on requirements of amino acids (3), vitamins (4–6) and phosphorus (7).

In some other instances, also, the principle has been applied, but often only empirically when, for example, it was found that using a semi-log plot of the data would produce conveniently straight lines. Among nutrition workers in general, application of the principle has only rarely been made, although it has great power in clarifying concepts of function and requirement.

The biological response to an increase in supply of some essential nutrient will very often be found to follow a curve of constantly diminishing increment, i.e., a logarithmic curve. In such cases, plotting the data on a semi-log basis will usually produce straight response lines up to the limit of response. Such a limit may be set by physiological, genetical or other non-nutritional limitations. At very low levels of nutrient intake, also, the data may be seen to deviate from the principle, but, in this case, largely because reserve stores of the nutrient in the animal or small residual quantities in the basal diet are no longer negligible in respect to controlled intake of the nutrient. When the limit of response is well defined, as by a plateau in the data, the minimal requirement then is readily perceived from the intersection of the response and plateau lines (2, 3, 5). Application and derivation of the principle has been discussed elsewhere (2, 4, 5).

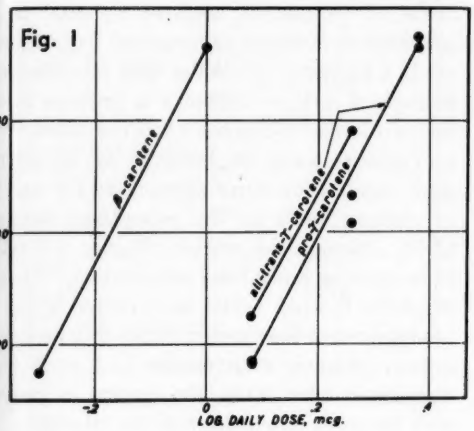
In the remaining time I shall present some examples of the applications of this Principle of Diminishing Returns as made from data existing in the literature. I will not cite the numerous further references, but will provide them to anyone who is interested.

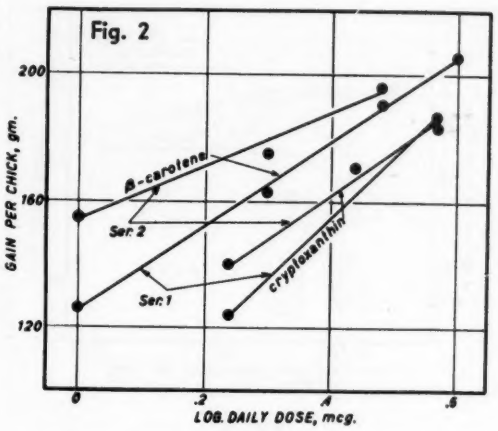
In figure 1 are shown the relations of rat growth to intake dose of several carotenes. The figure shows that the carotenes mentioned on the right were practically equivalent in potencies and constant in relation to the potency of β-carotene, over the range of the trials. The spacing between the lines is the logarithm of the potency ratio, in this case, approximately 2. This is rather typical of results obtained with different forms or precursors of a vitamin when these forms are very similar to each other.

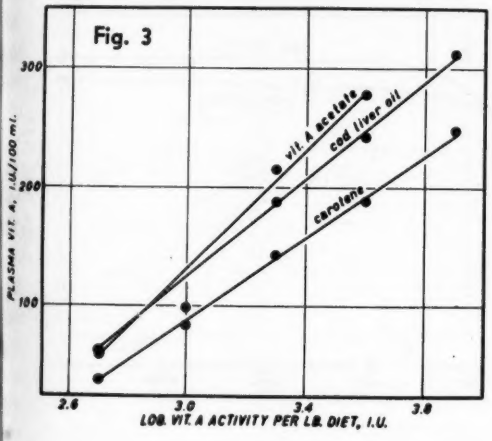
In the next figure is illustrated the fact that different forms or sources of a vitamin do not always show constant potency ratios (fig. 2). In other words, the attempts to evaluate one form of a vitamin in terms of another may in some cases be fruitless. The example shows that β-carotene and cryptoxanthin, as measured by chick growth, are not constant in relation to each other. This fact is fully consistent with the diminishing returns principle (4, 5).

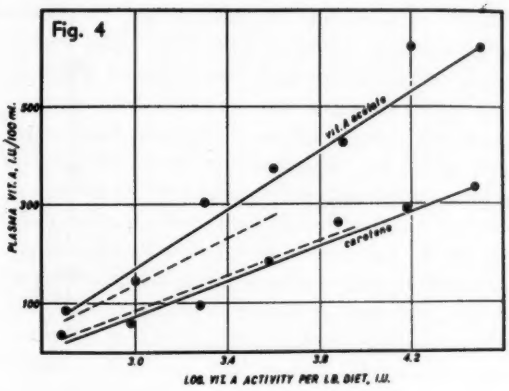
In figure 3 are shown data reported on the effect of three sources of vitamin A activity on the plasma vitamin A content of the chicken. It is again indicated that there is a systematic spread between the potencies, which increases as the intake. This feature is even more distinctly shown in figure 4, which represents the effects of carotene and vitamin A acetate on the plasma vitamin A of the turkey.

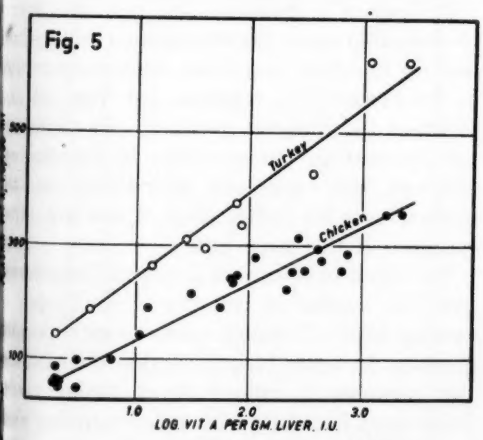
Figure 5 illustrates the relation of liver storage of vitamin A in these species to the plasma vitamin A. An interesting fact brought out by this











FIGS. 1–5

application of the diminishing returns principle that liver storage bears the same relation to blood vitamin A content as does the oral intake. Both sources appear to function in the same way the maintenance of the blood vitamin A level. This helps to explain why liver storage is fairly directly related to oral intake. The same relations of diet liver and blood levels may be observed in data from other animals such as the rat, sheep and cow.

Figure 6 presents some old but remarkably consistent data obtained with the rat. The growth response follows the typical straight line reaching a plateau. The plasma vitamin A content follows

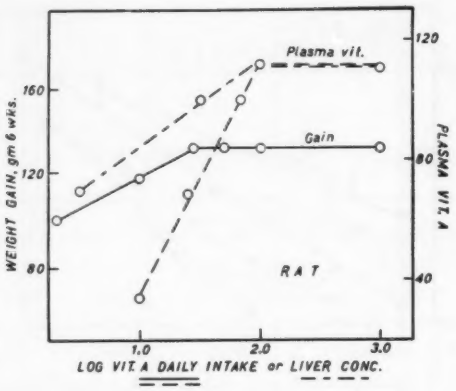


FIG. 6

forms of vitamin D, assayed by rats, on the calcification in chicks as measured by percentage ash in a leg bone. This set of data was cited as an example of lack of constancy in potency ratio of the two forms of vitamin D in the chick. When converted to a log plot, however (fig. 8), the data show that the two forms were practically constant in potency ratio in the suboptimal range of effect, although the weaker vitamin was unable to promote as much total calcification. This state of affairs is what would be expected if the less potent form of the vitamin enters into an enzyme system which is both weaker and more highly dissociated than when the system is provided with the more potent form of the vitamin.

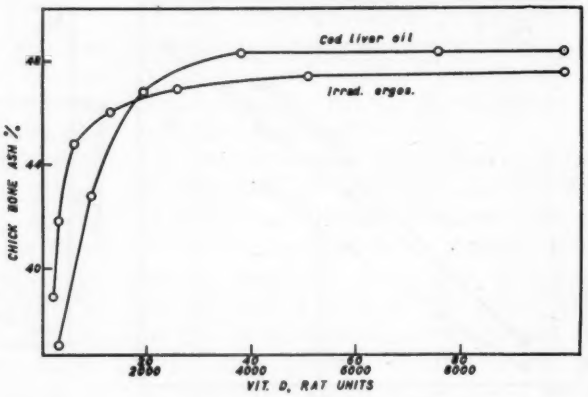


FIG. 7

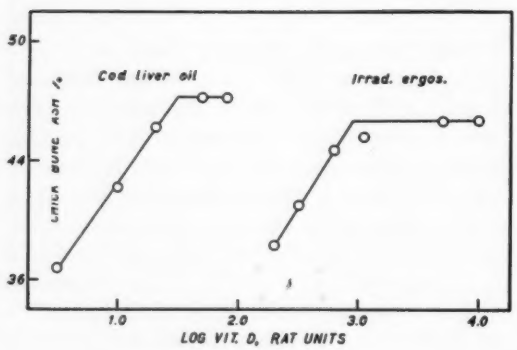


FIG. 8

a similar pattern. Furthermore, the plasma vitamin A shows a similar relation to liver vitamin A content as it does to oral intake.

Among the vitamins D are several interesting examples. Forms of vitamin D which are equivalent for rats may be very different in potencies for chicks. Some forms which are equivalent for chicks are, again, different for turkeys. In figure 7 is shown a usual type of plot of the effect of two

In figure 9 is illustrated the fact that forms of vitamin D which are standardized and equivalent for the chick may show different potencies in the turkey. The reference cod liver oil and Delsterol, a synthetic product, were definitely not constant in potency ratio in the turkey although they approach equivalence at the optimal dose for calcification. There are other examples of this same fact.

The effect of reserve of a vitamin transferred from the mother on response of the young to varying levels of vitamin intake is an important question. In figure 10 is illustrated the calcification responses to vitamin D of turkey poults which came from hens with widely differing vitamin D intakes. The effect of the hen diet on the poult response to vitamin D is clearly shown in the fanning-out of the suboptimal response lines. However, these remarkably precise data show a linear response from each class of poults and of All the response lines meet at the optimal vitamin calcifica intake of the poults.

Figure 11 illustrates the effect of an antibiotic is ev on lev

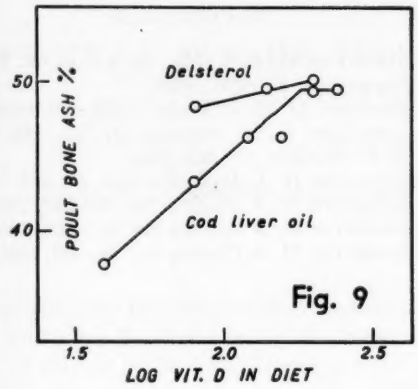
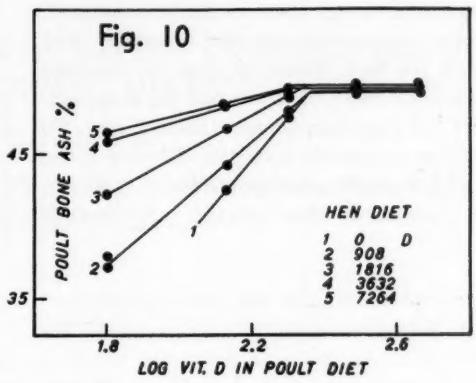


Fig. 9



FIGS. 9 AND 10

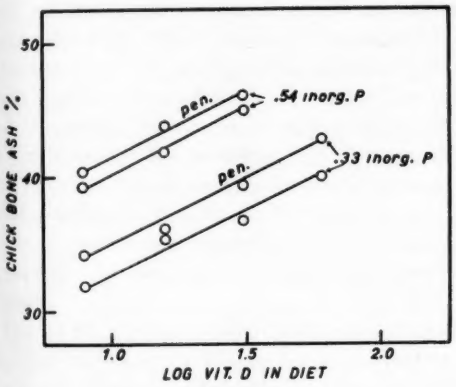


FIG. 11

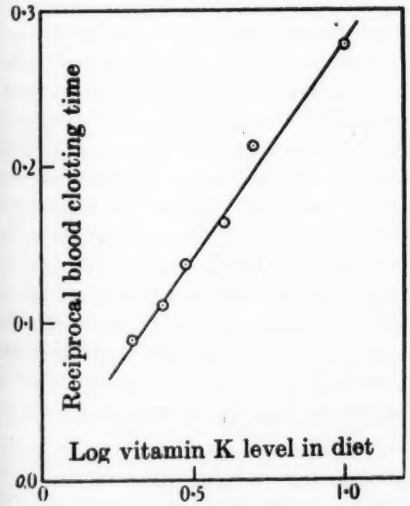


FIG. 12

nd of different inorganic phosphorus intake on
lcification response of the chick to vitamin D.
is evident that the penicillin antibiotic at the
w level of 2.5 grams per ton of feed distinctly enhanced the activity of the vitamin D, the effect being that of a constant potency ratio increase at each level of phosphorus. The phosphorus also increased the response from the vitamin D, as might be expected, since the lower phosphorus level was distinctly below requirement while the higher level was slightly above requirement. This effect of antibiotic in the enhancement of potency or absorption of an essential nutrient is now known to be quite general. The above example indicates that the effects were felt in this case to a uniform degree over the entire suboptimal test range. The writer has another example, not shown, in which the presence of bile salts in the diet was found to increase the potency of vitamin D by a constant ratio, indicating an improved absorption of the vitamin.

A number of examples are available in the case of vitamin K. Figure 12 shows an early expression of the relation of blood clotting power to the log of vitamin K level in the chick diet.

In figure 13 it is shown that a number of sets

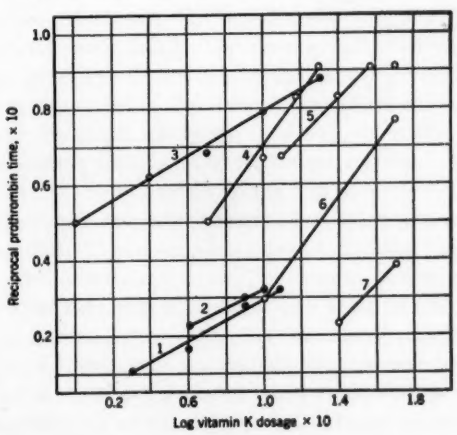


FIG. 13

of vitamin K data, when similarly presented, follow the diminishing returns principle. Also evident is the fact, shown by data all obtained from the same assay, that vitamin K_1, 2-methyl-3-phytyl-1,4-naphthoquinone, (*lines 5* and *7*), and another commonly used but different form, 2-methyl-1,4-napthoquinone, (*lines 4* and *6*), are not constant in their potency ratio to each other.

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EFFECTS OF VARIOUS TYPES OF LIPIDS IN EXPERIMENTAL HYPOLIPOTROPIC DIETS*

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CONSIDERABLE attention has been focused over the years, since the discovery of the lipotropic action of choline by Best and Huntsman (1), on the effect of variations of the dietary fat on fatty livers in rats fed choline-deficient diets. Less attention has been paid to the effects of varying the dietary fat under these conditions on tissues other than the liver. An attempt will be made here to bring together some of the knowledge of the subject, reviewing the available data as they apply to the liver and the effects of high-fat, low-choline diets on the cardiovascular-renal systems of similar animals. Finally, brief reference will be made to problems related to dietary cholesterol, lipotropic factors, and experimental arterial disease.

One of the earliest observations concerning the effect of variations of dietary fat on the liver was made by Hershey and Soskin in 1931 (2). They reported that the accumulation of abnormal fat in the livers of depancreatized dogs was greater if a saturated fat such as beef fat was added to the diet, than if a highly unsaturated fat such as cod liver oil was used. These investigations were later extended by Channon and Wilkenson (3), using rats fed a low-choline, low-protein diet. Under these conditions, the effects of high dietary supplements of butter fat, beef fat, palm oil, coconut oil, olive oil and cod liver oil were studied. The degree of accumulation of abnormal fat in the liver appeared to depend on the intake of C_{14}-C_{18} saturated fatty acids. With the exception of coconut oil, the severity of fat deposition varied inversely with the iodine value of the fat in the diet. A few years later an extension of these investigations was reported from the same laboratory (4), with the object of determining whether or not choline was concerned with the saturation of fatty acids in the liver. In this latter work, linseed oil was fed at a level of 40% either in its highly saturated, natural state or in progressively hydrogenated forms, reducing the iodine value as low as 55. The effect of varying the physical nature of the fat was further tested by adding elaidin in varying proportions to olive oil (elaidic acid being a solid isomer of oleic acid). The results again demonstrated that the amounts of fat deposited in the liver were inversely related to the iodine value of the different fats fed, and the extent of fatty infiltration was related to the proportion of C_{14}-C_{18} saturated fatty acids.

Stetten and Salcedo (5) continued these investigations 2 years later, and instead of focusing on the degree of unsaturation of the dietary fatty acids, as had the Liverpool group, they studied the effect of fatty acids in choline-deficient diets with respect to their chain length. They investigated the effect of each member of the homologous series of even-numbered saturated fatty acids from butyric to stearic. For a period of 2 weeks young rats were fed these acids as ethyl esters at the level of 35% of the hypolipotropic diet. By using the ethyl ester form, they avoided undue variation in the physical characters of the various fatty acids. The results indicated that the severity of the accumulation of abnormal amounts of fat in the livers of experimental animals increased markedly as the chain length of the dietary fatty acid decreased successively from 18- to 16- to 14-carbon atoms, but severely fatty livers were not encountered when fatty acids of less than 12 carbon atoms were fed under these experimental conditions. The results of Stetten and Salcedo, taken together with those of Channon and his group, indicated that the amount of liver fat that will accumulate in rats fed low-choline diets is influenced by both the degree of unsaturation of the dietary fat and also the molecular weights of the constituent fatty acids.

* Current investigations at Washington University, Department of Pathology, concerning the dietary production of vascular disease in experimental animals are being aided by a grant (H-1968) from the Public Health Service.

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Thus, the more saturated the fat in the diet and the greater the chain length of the dietary fatty acid in the range C_{14}-C_{18}, the greater the abnormal accumulation of fat in the liver. Stetten's group further encountered an unexpected finding in the hearts of the animals fed ethyl laurate in the choline-deficient food mixture, an observation that will be discussed below. Subsequently, in 1949, Campbell, Olley and Blewett (6), in agreement with the earlier investigators, demonstrated by feeding free fatty acids that palmitic and not oleic acid consistently produced fatty livers in adult rats fed low-protein diets.

A number of reports have come from India concerning the effects of varying the type of dietary fat added to both normal and hypolipotropic diets fed to experimental animals. The investigations of Damodaran and Siva Raman (7, 8) have been particularly noteworthy in this field. They found that coconut oil at a level of 15% or 25% in a choline-deficient diet composed of poor-rice (5% protein diet) was particularly effective in producing fatty livers in rats. Ghee (butter-fat) proved equally effective, whereas mustard and sesame oils fed at the same levels in similar choline-deficient diets resulted in very little abnormal accumulation of hepatic fat. Rather remarkably, livers of animals consuming diets containing 25% ghee became very fatty despite an appreciable drop in body weight. The Indian investigators further experimented with fractionated fatty acids, and demonstrated again that the accumulation of liver fat on low-protein diets is caused chiefly by the higher saturated fatty acids, although they could not explain the deleterious effect of ghee on growth in terms of its component fatty acids. These experiments accord with an earlier observation by Sveinsson (9) that diets containing 25–40% of cocoa fat fed to rabbits produced livers containing 30–40% of fatty acids. Later, Bose and Subrahmanyan (10) found that under the conditions of their experiments, ground nut oil and vanaspati, when compared with ghee, all fed at the level of 30% in low protein diets, failed to produce any significant difference in the fat storage, total cholesterol, or total fatty acids in the livers of rats under the conditions of their experiments. The Indian investigators did not describe the effects of the various dietary fats on lesions in organs other than the liver (i.e., kidney or cardiovascular system).

More recently, in this country Norcia and Lundberg (11) studied the effect of various synthetic simple triglycerides in diets fed to rats. These authors found that the relative amounts of mono-ethenoic acids found in the liver in their experiments appeared to be correlated with the amount of dietary choline. Compared to the effect of tripalmitin added to a choline-supplemented diet (1 gm choline hydrochloride/kg of diet), when this triglyceride was incorporated in a relatively low-choline diet, iodine values in the liver and body fat were 7–9 points lower, the mono-ethenoic acids were 9–17 percentage points lower, and saturated acids 8–15 percentage points higher. The authors concluded that these differences are correlated to the amount of available dietary choline. Their results should be considered in the light of recent investigations by Artom, to be discussed later.

György and Goldblatt (12), reviewing a large number of observations made over a period of 9 years on almost 2,000 rats, reported the effect of various fat supplements on the production of dietary cirrhosis. They noted that choline-deficient food mixtures containing lard (20–40%) produced a higher incidence of cirrhosis in the animals consuming the ration than in rats consuming similar diets incorporating hydrogenated fat (Crisco) at approximately the same level. These authors drew attention to the fact that in contrast to the incidence of hepatic abnormalities, renal lesions were more frequently found in rats fed choline-deficient diets containing Crisco than in those fed similar diets with lard. György and Goldblatt reported that cod liver oil (2%) in their experiments was especially injurious in choline-deficient diets, leading to the development of diffuse hepatic fibrosis, unaltered by supplements of tocopherol. This finding is perhaps rather surprising in view of Channon, Hanson, and Loizides' finding (4) that butter (IV 33) was associated with nearly six times the degree of abnormal accumulation of hepatic fat that developed in livers of rats fed diets containing comparable amounts of cod liver oil (IV 145), and also in view of the initial observation of Hershey and Soskin (2). In this respect it should be emphasized that cod liver oil and lard favor the deposition of the pigment ceroid in fibrous trabeculae of cirrhotic rats, and by its bulk ceroid may have produced an apparent increase in fibrous tissue. It should also be recalled that Crisco exerted a more damaging effect than lard on the kidneys of the rats in the experiments of György

and Goldblatt, and in this organ ceroid is not deposited in significant amounts. Accordingly, it is perhaps even more likely that ceroid accounts for the apparent differences between the fibrous and fatty effects of dietary fat on the liver. Perhaps other apparent discrepancies between the hepatic lipogenic and cirrhogenic effects of dietary fats in hypolipotropic diets can also be explained by the effect of the various fats on ceroid deposition in fibrous tissue under these conditions.

It was previously mentioned that Stetten *et al.* (5) encountered an unexpected finding in young rats fed a low choline diet containing ethyl laurate. Earlier investigators (13, 14) had shown that trilaurin supplements to normal food mixtures, when fed to rats, produce a depot of fat containing as much as 25% of lauric acid and, furthermore, this fatty acid is preferentially stored in the heart. Kesten, Salcedo and Stetten (15) discovered that their animals, fed 35–40% of ethyl laurate in their choline-deficeint diet, died within 3–6 days of a fatal myocarditis that greatly resembled the lesions produced by potassium deficiency. They carefully analyzed both their diets and the animals' hearts for potassium content, and failed to discover any evidence to suggest that potassium deficiency was responsible in any way for the myocardial lesions produced under these conditions by a hypolipotropic diet. Kesten *et al.* believed that the heart lesion was peculiar to animals fed only choline-deficient diets that contained lauric acid or one of its compounds. Stimulated by their report and by observations in Toronto at sporadic intervals of grossly evident necrotic lesions in the hearts of choline-deficient weanling rats fed a diet containing 12% beef fat, my colleagues, Professors Charles H. Best and George Wilgram, and I (16) were able to confirm and extend the observations of Kesten *et al.* (table 1). The results indicated that in susceptible animals maintained under severely hypolipotropic conditions, cardiac lesions appeared when a number of different types of fats were used in the deficient diet. Furthermore, not only were simple triglycerides effective in producing cardiac lesions in varying percentages of the experimental animals, but naturally occurring fats also exhibited a similar effect to greater or lesser degree. Lard and beef fat appeared more efficacious in this regard than coconut oil, although the Indian investigators had found the latter particularly effective in producing fatty livers when added to low protein diets at the level of 15% or 25% (8). It should also be recalled that although compounds of lauric acid lead to little abnormal fat deposition in the livers, they produce the most severe lesions in the hearts of choline-deficient rats, as observed by Kesten *et al.* (15). In a small percentage of animals, cardiac lesions were encountered even in the absence of any fat in a choline-deficient diet, and it was concluded that the dietary condition essential for the production of this type of cardiac necrosis is choline deficiency per se. The only dietary choline-deficient mixture that failed to produce cardiac lesions to some degree in the animals was one containing tributyrin. These animals all died within a short time with multiple gastric ulcers, as previously reported by Stetten *et al.* (5). Salmon and Copeland (17) have subsequently observed similar gastric lesions in rats fed tributyrin. These lesions are not preventable by choline. It is possible that if the animals had not died from the gastric lesions and had lived longer, even with tributyrin cardiac lesions might have been observed.

TABLE 1

Type of Fat, (25–40%)	No. Rats	No. With Cardiac Necrosis*
Ethyl laurate	25	15 (60)
Tributyrin	12	0
Tricaproin	13	6 (46)
Tricaprylin	12	7 (58)
Trilaurin	15	9 (60)
Trimyristin	12	3 (25)
Tripalmitin	12	2 (17)
Lard	82	53 (54)
Beef fat	10	8 (80)
Corn oil	10	4 (40)
Coconut oil	29	2 (7)
Fat-free	13	2 (15)

Condensed from data published in more detail previously (16).

* Figures in parentheses are percentages of the experimental groups.

Many of the animals in these experiments exhibited varying degrees of the hemorrhagic renal syndrome. The question arose whether or not the heart damage could be regarded as a direct result of choline deficiency on the myocardium, or as secondary to renal lesions induced by the hypolipotropic regimen. However, in a total of 25 animals, cardiac lesions were encountered in the absence of either gross or microscopic renal changes in choline-deficient rats. Furthermore,

Salmon and Copeland (18) have observed lesions of the cardiovascular system in both choline-deficient chickens and dogs. In neither of these species does choline deficiency produce significant renal damage. The manner in which choline deficiency may exert its deleterious effect directly on cardiac muscle will be considered later.

The pathogenesis of the cardiac lesions serves to focus attention on probable etiologic mechanisms, and has been published in detail elsewhere (16).[2] Kesten *et al.* failed to find any stainable fat in the hearts of their deficient animals, despite the application of suitable histochemical procedures to their material. In nearly all the animals in our experiments various amounts of stainable fat were demonstrated in the affected regions of the hearts. The greater the degree of necrosis, however, the less the amount of fat; the implication being that as the fatty myocardial fibers became necrotic, the fat was liberated and carried away by macrophages. As the animals of Kesten's group were examined at the stage of very advanced degrees of cardiac necrosis, this fat had perhaps already been removed by macrophages in their cases. Sudanophilic material in our animals appeared first within cardiac muscle fibers in linear arrangements of droplets following the muscle-striations. As the fat accumulated, muscle fibers became swollen and exhibited all the classical signs of degeneration, both nuclear and cytoplasmic. Prenecrotic and necrotic changes rapidly developed with lysis of fibers, dissolution of their cytoplasm and nuclei, and the areas of necrosis became invaded by an assortment of macrophages. Healing was characterized by collapse of the surrounding unaffected stroma around the area of necrosis and lysis, with shrinkage of the original area of the lesion. Proliferation of fibroblasts and sarcolemmal cells replaced the necrotic debris, producing a final picture of diffuse myocardial fibrosis. The latter lesion was present in animals fed the experimental diet for many months.

It is possible that the appearance of stainable fat in these hearts can be related to a decrease in the rate of fatty oxidation in choline deficiency. Artom (19, 20) has demonstrated that injection of choline into choline-deficient rats shortly before removal of tissues produced a greater rate of oxidation of fatty acids in slices of liver, kidney and heart *in vitro*, than in controls. Increases in production of radioactive $C^{14}O_2$ following administration of choline were observed in brain occasionally, never in testes. These findings lend support to the suggestion that choline deficiency may be the direct cause of the cardiac lesions. Several years ago Abdon and Borglin (21) reported the occurrence of bradycardia in choline-deficient rats. This phenomenon could be corrected very rapidly by injections of choline chloride. In their experiments, adult rats kept on a choline-free diet for 8–10 weeks frequently died very suddenly. The only sign before death was a bradycardia, the heart rate decreasing from the normal frequency of approximately 250 per minute to 70–80 per minute. Recovery, with a normal pulse rate, occurred within 10 or 15 minutes if choline was injected intravenously. Although these investigators could not determine chemically the amount of choline in hearts, they found that in skeletal muscles of young rats on choline-deficient diets, the amounts of muscle choline slowly decreased, and terminally a sudden fall in acetyl choline precursor developed. Further investigations by these authors demonstrated disturbances in the oxidative metabolism of muscle in choline deficiency (22). They found that minced muscle from the limbs showed a significantly lowered O_2 consumption. The addition of choline or methionine *in vitro* had no effect, but 1 ml of choline chloride injected intramuscularly 60 minutes before the animal was sacrificed restored the oxidative mechanism to higher than normal levels in the previously choline-deficient animals. In these experiments the drop in oxidative metabolism occurred in the animals before the onset of renal damage due to choline deficiency. More recently, the Alabama group of investigators (23, 24) have investigated choline deficiency in the rabbit and discovered a progressive muscular dystrophy in these animals, produced as a result of chronic choline deficiency and prevented by adequate supplements of this food factor. Although the results concerning skeletal muscle may not be directly applicable to cardiac muscle, nevertheless it is possible that dietary choline may play a role in the maintenance of normal striated muscle, whether of cardiac or skeletal distribution. These observations probably have no direct application to man

[2] A number of color transparencies illustrating the pathogenesis of choline-deficiency-induced cardiac lesions were shown at the oral presentation of this paper. Comparable black-and-white illustrations have been published in the reference cited, and are therefore not reproduced here.

although in one report fatty degeneration of myocardium has been described in over 40% of patients exhibiting centrolobular fatty change at autopsy (25).

In the course of the investigations at Toronto, described above, abnormal lipid in coronary arteries and aortic sclerosis were encountered in many of the young rats fed choline-deficient diets. These lesions were discovered most frequently in animals fed diets containing a high percentage (35%) of lard. The vascular lesions were usually seen in the animals that developed acute renal damage, and it is therefore entirely likely that the renal damage is a factor of considerable importance in the etiology of this type of experimentally induced coronary arterial lipoidosis and aortic sclerosis. However, similar vascular lesions, as already mentioned, have been encountered by the Alabama group (18) in choline-deficient chickens and dogs in which significant degrees of renal damage were rarely observed. The pathology and pathogenesis of the vascular lesions in rats have been reported in some detail (26, 27).[3] Whether or not the coronary arteries or the aorta are involved, the earliest abnormalities consist of the deposition of abnormal amounts of stainable fat in the intimal or subintimal tissues of the vessels concerned. In the aorta this initial lesion is followed by fatty degeneration and necrosis of the media, with eventual calcification producing a gross sclerosis.

The effects of varying the type of dietary fat in choline-deficient diets fed to animals under conditions that lead to vascular lesions have not been extensively investigated to date. Elsewhere at this meeting, the Toronto group (28) have reported that the addition of cholesterol supplements (2% for females and 1% for males) to a high-fat, high-protein, choline-deficient diet increased the incidence of cardiovascular lesions. Choline-deficient females otherwise resistant to the development of the lesions, when fed a cholesterol-supplemented, choline-deficient diet and injected with pituitary growth hormone (1 mg, 120-gm rat) and testosterone (5 mg, 120-gm rat) then developed cardiovascular symptoms of choline deficiency. It would appear, then, that not only lipid supplements but hormonal factors play a role in the development of these lesions. It is of interest that the serum cholesterol levels in choline-deficient rats are lower than in choline-supplemented controls, an observation previously made by earlier investigators (29). Even when added to normal diets without cholesterol supplements, choline and inositol increase the level of plasma cholesterol in chickens (30, 31). This fact, and observations on the effect of adding choline to cholesterol-supplemented stock diets, have led many investigators to believe that lipotropic factors do not play any significant role in the prevention of cholesterol-induced atheroma. But it should be emphasized that most of the experiments of this type (32, 33) have been carried out under conditions in which the normal diet contained adequate amounts of lipotropic factors to begin with, and the levels of cholesterol ranged from 2 to 5% or even more. Under these conditions it would be difficult indeed to demonstrate a lipotropic effect of added dietary choline even on the liver, much less the cardiovascular system. In monkeys, Mann *et al.* (34) have demonstrated a protective effect of methionine supplements to semi-synthetic diets containing added cholesterol. In preventing the development of atheroma in monkeys, the exact role of methionine vs. choline in this type of experiment is as yet not understood and is subject to further investigation and discussion (35). The effects of a few other dietary constituents on the production of choline deficiency-induced vascular damage in rats have been investigated. Hypervitaminosis D is much less harmful to the aorta and coronary arteries of rats given a supplement of choline chloride (1%) than when similarly excessive amounts of the vitamin (15,000–20,000* IU/day/rat) are added to the basal hypolipotropic diet (36). Tocopherol added to the basal diet exerted no effect on the development of vascular lesions, whereas betaine supplements completely protected the animals (37).

In conclusion, it is apparent that the effects of a lipotropic-deficient diet on a number of organs of the experiemental animal are probably highly influenced by the type of dietary fat and the quantity employed. These relations have been intensively investigated with regard to the liver, but as yet only the beginnings of the picture are emerging with reference to the kidney, the heart, and the vessels. There appears little doubt, how-

[3] A number of color transparencies were shown at the oral presentation of this paper illustrating the morphology of the lesions. Comparable black-and-white photomicrographs have been published in the references cited, and are therefore not reproduced here.

ever, that under suitable conditions in the laboratory inadequate supplies of lipotropic factors in the diet may produce dramatic and striking changes in a variety of organs in addition to the liver. The brilliant investigations of Abdon, Artom, Gurin (38) and others may point to a final common pathway in the pathogenesis of all these lesions by indicating the mechanisms whereby choline enables all these tissues to metabolise fatty acids at normal rates. We feel that suitable manipulations of the protein, choline and fat content of experimental diets in future investigations may yield highly significant and revealing results in the field of cardiovascular and renal investigation.

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HORMONAL-NUTRITIONAL-LIPID RELATIONSHIPS[1]

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A HIGHLY comprehensive title gives one the privilege, at the one extreme, of saying everything about nothing; and at the other, of saying nothing about everything. We shall try to avoid either extreme by limiting the discussion to a few facets in the broad field of lipid metabolism. Specifically, we shall confine our observations to some factors having to do with plasma lipid levels, and to a brief consideration of hormonal regulation of neutral fat metabolism.

RELATIONSHIP OF DIET TO LEVEL OF SERUM LIPIDS

In no field of clinical investigation is there more disagreement than in that dealing with the relationship of diet to plasma lipid levels, with particular reference to cholesterol. Approximately 40 years have elapsed since it was first noted that the feeding of cholesterol to a rabbit would produce hypercholesterolemia and vascular changes, suggestive of those found in human atherosclerosis (1). Many investigators have felt that the observations in the rabbit justified the conclusion that dietary cholesterol in the human was a potentially toxic substance. Others have felt that with or without cholesterol, dietary fat per se would predispose to elevated plasma lipid levels, and to abnormal deposition of lipids in the blood vessels.

The matter of the relationship of dietary fat to plasma lipid levels has been under investigation in our laboratory during the past several years. The initial observations were obtained inadvertently in the course of maintaining a patient on a diet in which all of the calories were derived from a vegetable fat emulsion. The study was designed to enable us to evaluate the effects of certain of the cortical steroid hormones upon fat oxidation. During the ingestion of this material, it was noted that the plasma cholesterol and phospholipid values in the first patient fell in rather striking fashion (2, 3). These findings have recently been confirmed by Ahrens *et al.* (4) and by Beveridge *et al.* (5). Our initial interpretation was that this change was attributable to some of the hormonal material which was being administered at the time. Subsequently, however, it was found that in the absence of any hormonal therapy, the administration of diets containing large amounts of fat (the fat all being of vegetable origin) would consistently result in a very profound decrease in the level of plasma cholesterol, this decrease averaging approximately 100 mg per 100 ml in normo-cholesterolemic individuals, and several times this decrease in hypercholesterolemic individuals (figs. 1 and 2). When isocaloric amounts of animal fat were substituted for the vegetable fat, a prompt rise in plasma cholesterol occurred to or above the original level. It appeared, therefore, despite a few reports to the contrary in the literature (6, 7) that animal fat and vegetable fat were essentially different substances insofar as their relationship to plasma cholesterol and phospholipids was concerned.

These observations raise certain questions, among which are the following:

Are the changes in plasma cholesterol related to a simple lack of cholesterol in the vegetable fat? The full answer to this is not yet forthcoming, but it seems probable that other factors play a part, inasmuch as the addition of large amounts of cholesterol to the high vegetable fat formulae has not, under the experimental conditions in which it has been used in our laboratory, resulted in a rise in plasma cholesterol (8).

Is the effect attributable to some positive factor in the vegetable fat; e.g., some phospholipid or vegetable sterol? We have attempted to evaluate a number of such vegetable fat derivatives. A decrease in plasma cholesterol has been observed with some of these, but the results have been erratic, and in no instance has the magnitude been that obtained with the high vegetable fat intake (9).

Is the effect attributable to nutritional defi-

[1] This work was supported in part by grants from the National Institutes of Health, The Armour Laboratories, The Upjohn Company, Schering Corporation, Merck & Company, and the Alameda County Heart Association.

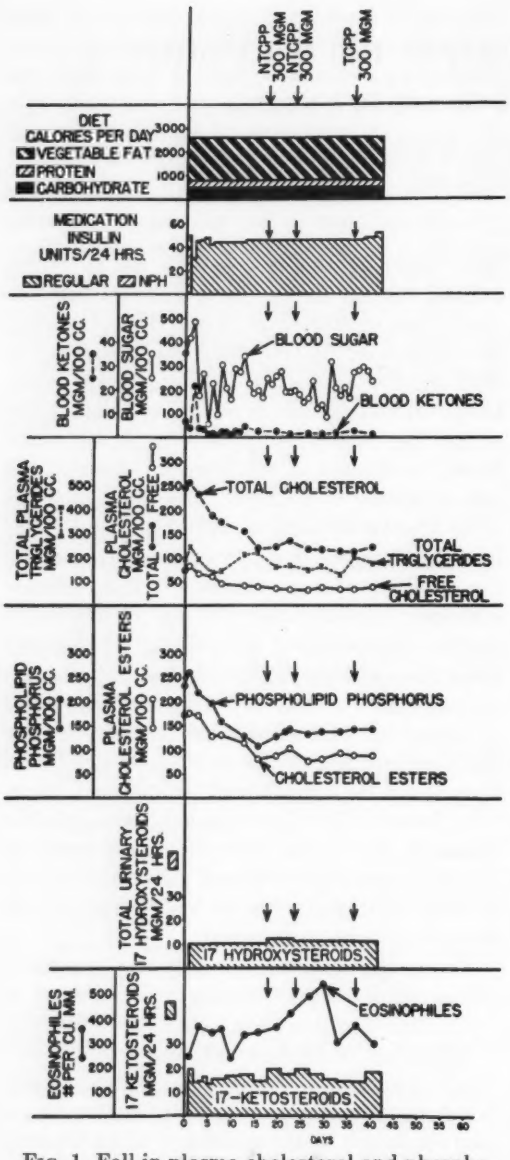

FIG. 1. Fall in plasma cholesterol and phospholipids during intake of a high vegetable fat diet in a juvenile diabetic. (*Patient CART*, 28 yr., diabetes mellitus, Sept. 28–Nov. 9, 1954.)

ciency brought about by an improperly balanced formula? This interpretation has been advanced in a recent paper in *Nutrition Reviews* (10). That this is not the case is readily shown by the following: *a*) Patients have been maintained on these formula diets in positive protein balance and in weight equilibrium or actual weight gain over prolonged periods of time (refs. 11, 12, and fig. 3). *b*) Effects of equal magnitude can be obtained when one uses mixed diets, thoroughly

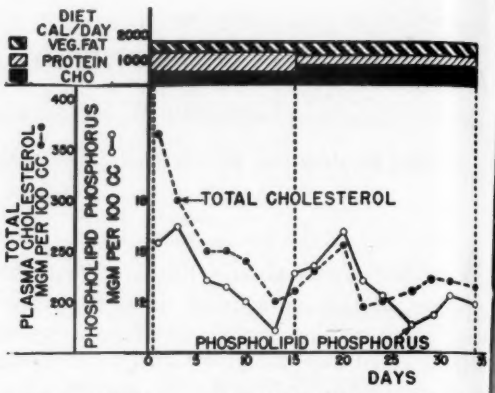

FIG. 2. Fall to normal of pathologically elevated plasma cholesterol and phospholipids during intake of a formula diet in which all the fat was of vegetable origin. (*Patient DID*, 28 yr., diabetes mellitus, Nov. 11–Dec. 15, 1952.)

adequate in all known nutritional entities, in which a large portion of the vegetable fat and of the protein is derived from a variety of nuts (fig. 4). Additional data bearing on this aspect of the problem are included in a recent report from this laboratory (13).

Could the unsaturated fatty acid content of the fats used play a part in the effect upon serum lipids? All of the vegetable fats used by us initially have had very high iodine numbers. The unsaturated fatty acid content of animal fat averages less than half of that of vegetable fat. Currently under way is a study in which a vegetable fat having a very low iodine number is being used on a comparative basis (see ADDENDUM).

LIPID METABOLISM IN RELATION TO ATHEROSCLEROSIS

This field has been the subject of a great number of papers and multiple reviews. A recent summary of the subject by Friedman, Rosenman and Byers approaches the problem objectively (14). We would like only to make the following comments.

The etiology of atherosclerosis is unknown. That it may be nutritional is entirely possible. Should this be so, it is equally possible that the actual nature of the nutritional deficiency or excess is still unsuspected.

There is as yet no laboratory procedure which in any sense of the word can be considered as 'diagnostic' of early atherosclerosis. Also, this is true of many patients with extensive atherosclerosis which is not yet clinically evident. This statement applies to chemical determinations of blood lipids; to lipoprotein patterns

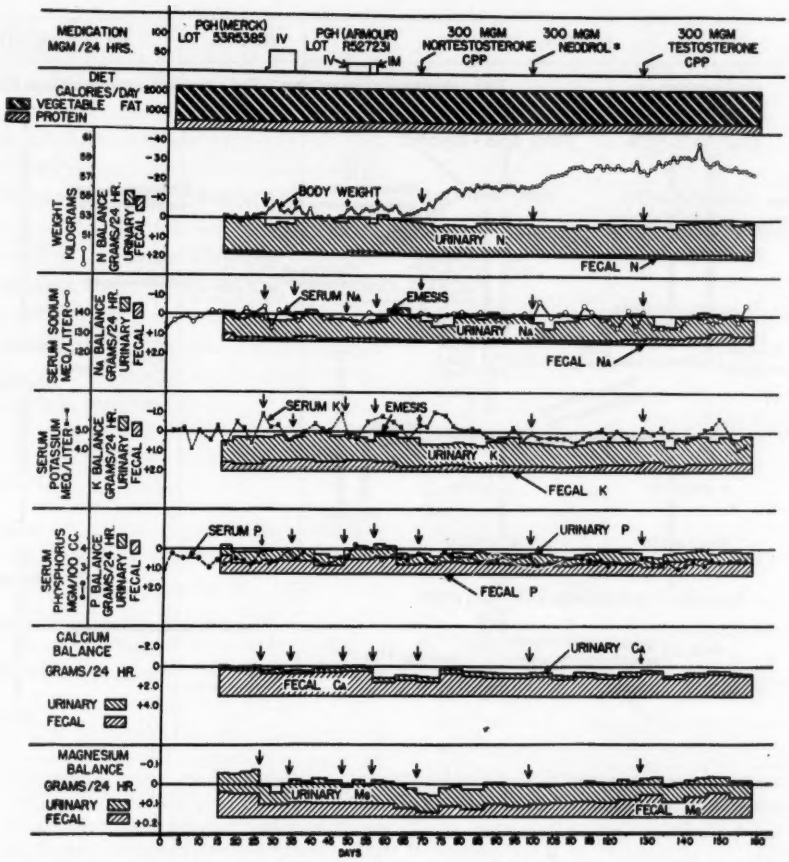

FIG. 3. Maintenance of positive protein balance in an elderly patient receiving a formula diet containing only vegetable fat and protein during a period of more than 5 months. (*Patient DUT*, osteoporosis, Jan. 6–June 13, 1954.)

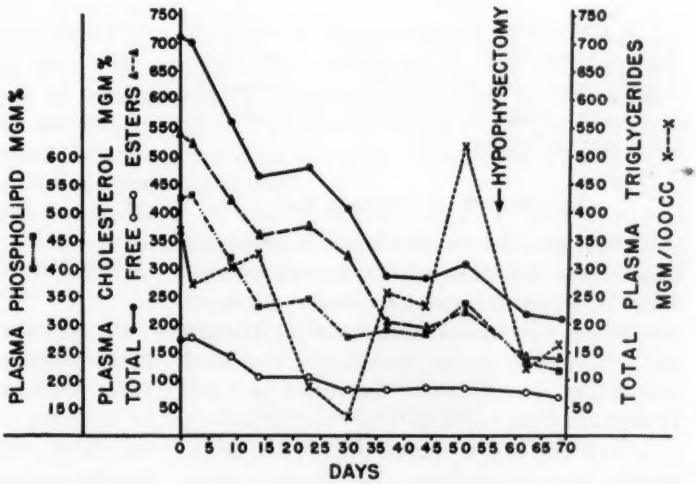

FIG. 4. Striking fall in plasma cholesterol and phospholipids in an initially hypercholesteremic diabetic. During this period of study, he was maintained on a mixed well balanced diet containing large amounts of fat, all of vegetable origin. (*Patient FAW*, 37 yr., diabetes mellitus, July 21–Sept. 27, 1954).

however obtained; and to 'atherogenic indices' resulting from unusual mathematical manipulations of some of the above chemically or physically determined plasma or serum constituents.

On the basis of life insurance company statistics, there appears to be little question that the avoidance of obesity represents a prophylactic procedure of some value.

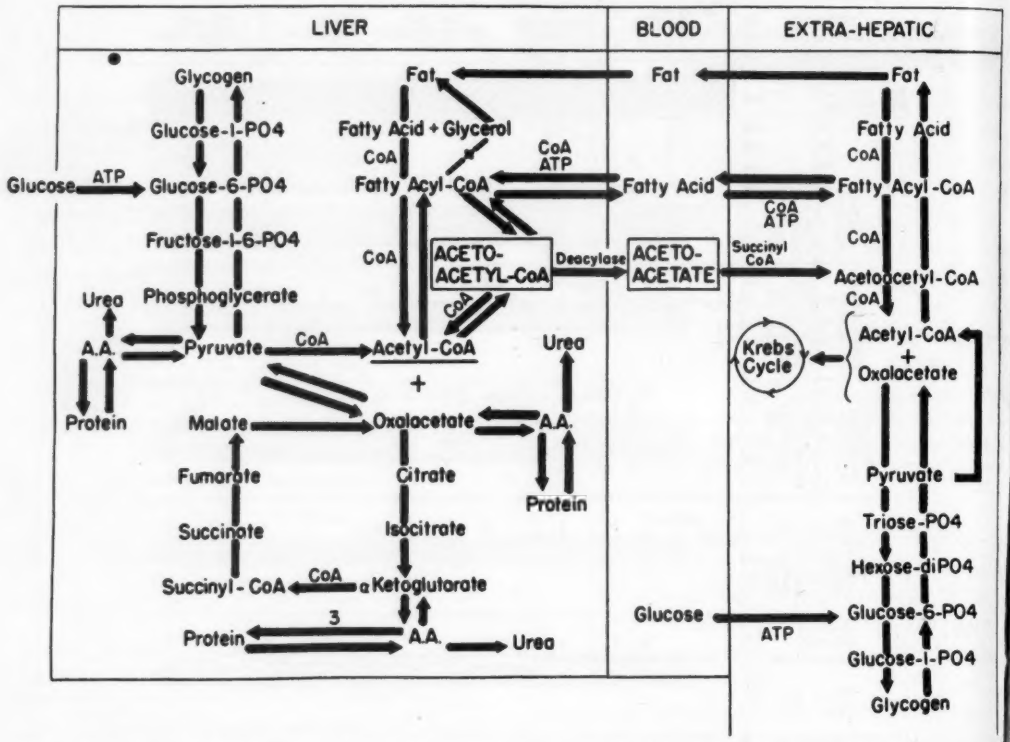

FIG. 5. Concept of fat oxidation in the human subject. Certain factors deserve particular emphasis: *1*) Activated acetate is the central hub of all energy metabolism. *2*) Oxalacetate and/or other members of the Krebs cycle are derived from pyruvate, and hence from either protein or carbohydrate. *3*) Lacking a sufficient supply of precursors of the members of the Krebs cycle, activated acetate from fatty acids 'piles up' with resultant formation of acetoacetate. In this sense "fat burns in the fire of the carbohydrate." *4*) Insulin, by directly or indirectly regulating the Krebs cycle, increases both carbohydrate and fat oxidation. (Diagram used by permission of Dr. F. L. Engel.)

Our therapeutic armamentarium is extremely impoverished. Until this situation is improved, it seems reasonable to us to believe that adherence to a course designed to avoid hypercholesterolemia may be sensible.

METABOLISM OF NEUTRAL FAT

Knowledge of fat metabolism has accumulated more slowly than that relating to either carbohydrate or protein metabolism. Some reasonably solid pieces of information are available, although the total picture is still incomplete. The most stimulating observations of the recent past and present are those which indicate that fat may be considered to be the 'prime fuel' of the body, rather than carbohydrate (15). Equally interesting, is the return to a modified version of the old concept that "fats burn in the fire of the carbohydrate" (16, 17). Both these concepts are brought out in figure 5.

HORMONAL REGULATION OF LIPID METABOLISM

For most purposes, a hormone may be considered to be an agent which accelerates or inhibits the rate at which an enzymatic process occurs. This will be the approach used in discussing our very incomplete knowledge of the effects of hormones on lipid metabolism.

HORMONAL REGULATION OF CHOLESTEROL AND PHOSPHOLIPID METABOLISM

For all intents and purposes, nothing is known in this field, insofar as mechanisms are concerned. It has been shown, however, that certain states of hormone excess and hormone deficiency are associated with elevation or depression of the plasma cholesterol level; i.e., *a*) the level of thyroid hormone administration or production is inversely related to the level of plasma cholesterol (18, 19). *b*) Chronic hypoinsulinism (diabetes) tends to be associated with hypercholes-

terolemia and hyperlipemia generally (20). *c*) Increased adrenocortical activity in response to corticotropin administration may be associated with a decrease in plasma cholesterol (21). *d*) Administration of pharmacologic amounts of cortisone and related steroids may be associated with elevation in plasma cholesterol (22).

Generally speaking, factors which cause elevation or depression in plasma cholesterol produce the same type of effect upon plasma phospholipids. Presumably, this means that free and esterified cholesterol and lecithins tend to occur in a reasonably constant relationship in the plasma lipoprotein complexes.

EFFECTS OF HORMONES UPON METABOLISM OF NEUTRAL FAT

Pituitary Factors. Since no anterior pituitary hormone has as yet been isolated in 'pure' form, it is obvious that one can speak with little assurance about precise quantitative effects of such hormones. There is suggestive evidence that a material called 'adipokinen,' which has not yet been separated from quite highly purified corticotropin, tends to mobilize fat from the liver to depots, and possibly to indirectly accelerate fat combustion (23). The non-ACTH diabetogenic factor (which has not yet been separated from pituitary growth hormone) directly or indirectly also appears to have a significant effect upon fat metabolism, the nature of which remains to be clarified (24).

Thyroid Hormone. This material accelerates the rate of all metabolic processes including fat mobilization and oxidation.

Insulin has a two-fold effect upon fat metabolism—it increases the formation of fat from carbohydrate (25), and indirectly decreases the formation of ketones from fat via its effect upon carbohydrate metabolism (26; see fig. 5).

Steroids. *Cortisone-like steroids* decrease the hyperketonemia produced by starvation and high fat feeding (27).

Anabolic steroids, directly or indirectly, may increase the rate or efficiency of fat catabolism in association with increased protein anabolism (28).

SUMMARY

It is well established that the ingestion of large amounts of a variety of vegetable fats, either in the form of a nutritionally adequate formula, or a mixed diet, in the complete or nearly complete absence of animal fat, results in profound decrease in plasma cholesterol and phospholipids. The mechanism of this effect is still unclear. Whether the decrease in these plasma lipids is of any value, in terms of prophylaxis or treatment of human atherosclerosis, is still to be determined.

The knowledge of oxidation of neutral fat, and the place of fat in the 'metabolic pool,' has become much more precise. To a lesser degree this is true of our knowledge of hormonal regulation of lipid metabolism.

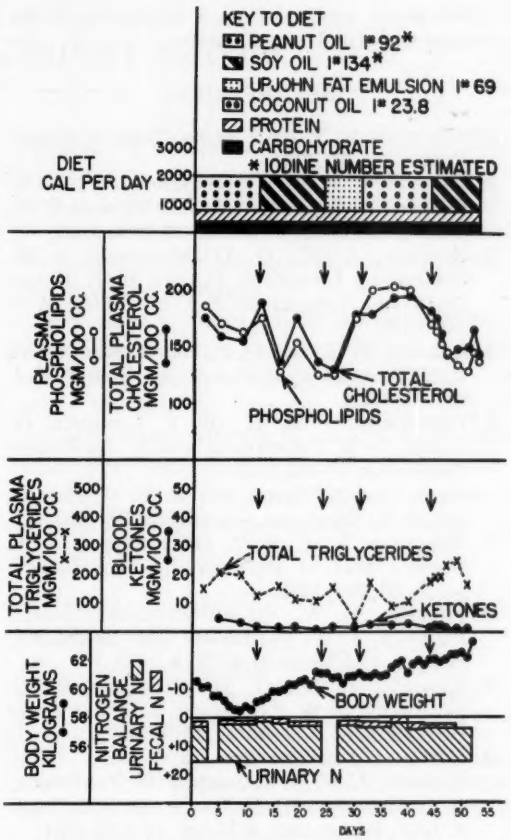

FIG. 6. Relationship between unsaturated fatty acid content of the diet and the level of plasma cholesterol and phospholipid. (*Patient AING*, 30 yr., diabetes mellitus, Dec. 12, 1954–April 1955.)

ADDENDUM

In figure 6 are shown the results of a recent study in which calorically equal amounts of *vegetable* fats of high and low unsaturated fatty acid content, respectively, were administered at intervals during the course of a 'balance study'. It appears that the fall in plasma cholesterol is directly related to the unsaturated fatty acid content of the fat used. Both fats are essentially cholesterol-free.

This study resulted from a discussion of the problem with Dr. Harry Deuel.

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FEDERATION PLACEMENT SERVICE

FOUR YEARS AGO a short description of the development and activities of the Federation Placement Service was prepared for *Federation Proceedings*. This included a brief analysis of the experience, qualifications and requirements of the candidates and of the types of positions registered by employers. Such a study led to some modifications of the Service designed to meet more fully the needs of the two groups using it.

During the ensuing interval the number of candidates on the rolls during one calendar year increased from 453 to 792 in 1953, or an increase of 74%. There was a small decrease of about 5% in 1954. Since the totals do not represent a calendar year but a March 1 to March 1 tally, the 1954–55 figures are probably affected by the location of the 1955 annual meeting at a point too distant for many of the graduate students from the east who are about to receive degrees to attend. This would decrease the number from the east registering in the months preceding the meetings, who do so with the primary purpose of utilizing the interview facilities there. It has been these recent graduates of the eastern and midwestern schools who have made up the largest group of candidate registrants. The totals may also reflect a changing employment situation .with more desirable positions open.

Since the lists have been placed on a subscription basis, fewer positions have actually been registered, for employers no longer must register a position to receive the list of candidates. If they do not wish an opening publicized they may prefer to subscribe to the list of candidates without specifying what positions they have to fill. Also, regular subscribers have in their files the current list of candidates to consult when an opening occurs and negotiations for a replacement may be begun without direct consultation with the Service, particularly if the next issue of a list of positions is some months away. Therefore, any analysis and comparison of positions for the two years under study would be of little value.

There has been a steady increase in the number of employers using the interview facilities at the annual meeting. In 1954 there were 175 employers representing 125 of the 285 subscribers eligible to use this part of the Service. The total number of annual subscriptions has been between 250 and 275 with an average increase of 10–15 subscriptions each year. The number of single-copy orders has doubled in that time. Many employers find it best meets their needs to purchase the February list alone, since this carries convention interview privileges.

In an effort to meet partially the increased cost of handling the larger number of individuals now using the Service and the growing costs of providing satisfactory interview facilities at the annual meetings, the list of candidates, of positions and of fellowships have been on a subscription basis for 3 years. The Federation Board voted in April 1954 to increase the fees beginning with 1955 so that at present they are:

Annual subscription	$15.00
Single copy order of	
List of candidates	10.00
List of positions	5.00
List of fellowships	2.00

A copy of the fellowship list is sent without cost to any member of a constituent Society, upon written request. To utilize the interview facilities at the annual meeting the employer must be either an annual subscriber or his authorized representative or have purchased the February list of candidates on a single-copy basis. The candidate pays an annual fee of $3.00 to maintain his registration. Such fees enable the Service to meet approximately half its cost, the Federation bearing the remainder.

As the last analysis of the candidates using the Service and of the types of positions offered by employers is now 4 years old, it has seemed of interest to prepare another such study and a comparison of the two. A comparison of the candidates of the two years is more meaningful than an attempt to compare the incomplete figures on positions which we have, because so few of the positions actually recruited for through the Service are registered.

The numbers of candidates from any one field do not correspond to the relative size of the society representing that group. The American Physiological Society is the largest, with 1,423 members; the American Society of Biological Chemists is second with a membership of 1,104,

TABLE 1. TOTAL APPLICANTS REGISTERED MARCH 1 TO MARCH 1

Field	1950–51	%	1954–55	%
Physiology	173	38.3	247	33.3
Biochemistry	214	47.3	335	45.2
Pharmacology	38	8.4	71	9.5
Pathology	7	1.6	10	1.3
Nutrition	10	2.2	23	3.1
Immunology	2	.4	55	7.4
Misc.	8	1.8		
Total	452		741	

TABLE 2. APPLICANTS AND POSITIONS REGISTERED, ATLANTIC CITY MEETING, APRIL 1954

Field	Applicants Reg.	No. Receiving Interviews	No. Interviews	Positions Reg.
Physiology	103	74	226	47
Biochemistry	184	111	316	65
Pharmacology	27	18	86	24
Pathology	2	2	4	5
Nutrition	14	8	14	1
Immunology	14	7	8	2
Total	344	220	654	144

but its membership has increased more rapidly the last few years, seeming to indicate more young men are entering this field. The American Society for Pharmacology and Experimental Therapeutics is third, with 627. As in 1950-51, more biochemists used the Service than any other group, and it is interesting that the candidates of the two years which are being compared represent the fields of the Societies in about the same proportions, as is seen in table 1. This proportion has remained remarkably constant at any time a tally was made. This is evident in table 2 where the candidates and the positions registered at the annual meeting are listed according to their field of interest. Not only do biochemists register in larger numbers, but more positions are registered in that field and more interviews requested. But a larger percentage of physiologists received interviews than did biochemists in 1954 (table 2).

Again, a brief study of the qualifications of candidates who received interviews was made. Each year about $\frac{2}{3}$ of the candidates receive interviews while $\frac{1}{3}$ receive no requests.

It was found earlier that candidates registering the first day of the registration period, and even until noon of the second day, had the best chance for interviews. This seemed to have little or no relation to their qualifications. Since a candidate's records cannot be put in the open files until he completes a time card indicating when he will be available for interviews, these records cannot be studied by employers until the candidates sign in with the Placement Service at the annual meeting. If they are not in the open files until the third day an employer may not see them, should he make only one visit to these files and not return to look over later registrants. Records are filed in order of registration and in numbered books to facilitate subsequent visits by employers. Certain employers are careful to go over all applications in the field, but apparently the majority do not, finding too many demands made on their time by other events. Also these same employers may wait until the last day to schedule the appointments they wish, in some cases to find the candidate, discouraged by no interviews, has gone home a day or even two days earlier than he had intended.

To eliminate this difficulty, both candidates and employers are encouraged to use the list of candidates prepared in February and mailed to subscribers about 6 weeks before the annual meeting. In 1954, when good advance publicity concerning this list was placed in *Federation Proceedings*, an unusually large number of candidates registered in time to be entered in the list. This gives the employer time to study their qualifications at leisure and to reach tentative decisions on whom he wishes to see, providing of course that these candidates attend the meeting. In table 3 it can be seen that the percentage of candidates who received interviews was larger in the group described in the February list than in the total registration. They also received more interviews per individual.

In the same way, the March list of positions reaches all subscribers several weeks in advance of the meeting. Employers who wish to be approached by candidates can enter their open positions in this list. There is then ample time for

TABLE 3. NUMBER ON FEBRUARY LIST WHO REGISTERED AT ATLANTIC CITY

		No. Received Interviews	% Received Interviews	Av. No. Interviews /Person
Total registrants	345	221	64	3.0
No. of registrants on Feb. list	132	98	74	3.6

TABLE 4. APPLICANTS INTERVIEWED

	1951			1954		
	No. Applicants	No. Interviews	Interviews/Person	No. Applicants	No. Interviews	Interviews/Person
Ages						
20-25	15	61	4.1	17	56	3.3
26-30	79	321	4.1	129	382	2.9
31-35	38	95	2.5	63	198	3.1
36-40	19	46	2.4	7	14	2.0
41-45	3	6	2.0	3	3	1.0
Over 45	2	3	1.5	1	1	1.0
Totals	156	532		220	654	
Degrees Held						
None					1	
A.B. or B.S.	5	22	4.4	8	13	1.6
M.A. or M.S.	16	42	2.6	21	60	2.8
Doctorate	130	438	3.4	186	560	3.0
Ph.D. & M.D.	5	30	6.0	5	20	4.0
Totals	156	532		220	654	

terested candidates to write to them before the eetings, where an interview can then be arnged by the employer.

As in the previous study, the greatest number f applicants who received interviews are in the 6-30 age group (table 4). These individuals ave received their doctorates recently, but may ave a year or two of experience. The next igher age group, 31–35, received more interviews er person than in 1951. This substantiates the mpression that a trend has been developing mong employers now to seek candidates with ome postdoctoral experience. Another factor ay be the increased availability of desirable esearch fellowships affording postdoctoral exerience in the sciences. Thus a candidate may ot need to seek a permanent position immeditely upon receiving his degree. In 1951 the ar-induced shortage of new graduates was still pparent. The slight differences shown by these ables can hardly be called significant, however.

A breakdown of the degrees held by applicants ontinues to show an overwhelming majority ith doctorates and the greatest number of nterviews going to such candidates (table 4). nce the Service is designed to meet the needs of is group this would seem to be some measure of s success in doing so.

A summary of asking salaries of the candidates gistered at the annual meeting in 1954 is found table 5. No record is obtainable of the salaries received, since we have very inadequate reports on the positions candidates accept. These reports are on a voluntary basis, as no fee on actual placement is involved, and only a minority of candidates cooperate. In general, salaries have risen $1,000–$1,500 per year in the past 4 years for all levels of preparation and experience on which there is sufficient information to make a general statement.

The great increase in numbers of individuals using the Service in these years clearly demonstrates the need for it. The rate of increase has lessened somewhat in the past 2 years. This may reflect a changing employment situation, as mentioned previously, or may indicate that the Service as now conducted has reached most of those who wish to use it. Certainly its usefulness to candidates for major positions could be increased. However, those positions must be handled on an individual basis, which is not possible with the present staff. Such an adaptation would add substantially to the cost of operating the Service, and would constitute a major policy change.

Since we have inadequate information about the positions for which employers are recruiting, a summary of the employers who subscribe to the

TABLE 5. SALARIES ASKED AT ATLANTIC CITY, 1954

Salaries	Applicants		Total
	Non-interviewed	Interviewed	
Open	18	41	59
Under 3000	1		1
3000–3900	4	8	12
4000–4900	13	42	55
5000–5900	37	86	123
6000–6900	28	38	66
7000–7900	11	2	13
8000–8900	8	2	10
9000 & over	4	1	5
Totals	124	220	344

TABLE 6. 1954 SUBSCRIPTIONS

	Annual	Single	Total	%
Universities	160	30	190	60.7
Governmental	17	6	23	7.3
Research Inst.	25	3	28	8.9
Industrial	44	6	50	16.0
Personal	19	3	22	7.0
Total	265	48	313	

Service and the type of institution they represent might be of interest. This is presented in table 6.

It will be noted that 60% of the subscribers are from universities, where departments can make use of both the lists of candidates for filling any openings and the lists of positions for their students who are seeking employment opportunities. Obviously the other classifications are in general interested only in the list of candidates.

The annual list of fellowships and assistantships has now been issued four times and covers most of the well known fellowships in the field of experimental biology. Excellent cooperation has been received from the larger granting organizations. This list is designed to give the information necessary to an individual wishing to apply for the fellowship. It includes: the purpose for which the fellowship is granted, the name of the granting organization, address for application, requirements for applicants, stipend, final date of application and of award. This could be considerably more useful with an increased registration of the less well known fellowships, particularly those granted by universities. As universities differ widely in their procedures in granting such fellowships and they in turn change from time to time, it is difficult to reach the person who has the responsibility for such registration. Members of the Federation who hold academic positions could give valuable assistance by taking the initiative in registering the fellowships and assistantships available in their departments.

LETHA K. ANDERVONT
Assistant to the Director

Placement Service 1955 Federation Meeting

The Placement Service interview facilities at San Francisco were located on the second floor of the Civic Auditorium where a generous amount of space was available. As had been anticipated, the number of candidates using the interview service this year was considerably reduced. A total of 165 candidates registered. Of these 128 or about 80% received interviews, a considerably higher percentage than in previous years. Also of the total 165, 98 or 59% indicated biochemistry as their major field of interest.

There were 116 employers registered representing 85 subscriptions and 406 interviews requested. Compared with other years this shows a proportionally greater attendance of employers than candidates, with a consequent greater number of applicants receiving interviews and more interviews per applicant.

CORRECTIONS AND ADDITIONS TO MARCH ISSUE

Page 7. THEODORE A. BALOURDAS, *lines 34–36* should read as follows: ". . . showed evidently favorable influence upon the capillary permeability; slight or none, in other experiments, upon the increased epinephrine sensitivity."

Page 79. A. C. IVY, *3rd line* should read: "Aminoguanidine HCl (10 mg/kg body wt.)."

Page 100. *Author's name and address* should read: "MILTON MENDLOWITZ. *Dept. of Medicine and the Andre Meyer Dept. of Physics, Mount Sinai Hosp., New York City.*"

Page 113. E. R. PERL, *12th line*, following ". . . flexion," add the following sentence: "In decerebrates, a discharge to crossed extensors (if present) appeared after 30–50 msec." *Line 25*, following "earlier," insert the words "onset of."

Page 117. RAAB AND GIGEE. Add the following acknowledgment of assistance: ". . . and by Am. Med. Assoc. Grant No. 87."

Page 133. LEON H. SCHNEYER. The following revised abstract should be substituted for that printed:

Source of total mixed saliva of man. LEON H. SCHNEYER (introduced by JOHN M. BRUHN). *Depts. of Physiology and Pedodontia, School of Dentistry and Med. College of Alabama, Univ. of Alabama, Birmingham.*

Recent work indicates that, under essentially 'resting' conditions, the volume flow of the submaxillary, sublingual and parotid gland secretions accounts, on an average, for 58% of the volume flow of the total mixed saliva of man, thus leaving the origin of 42% of the total mixed saliva unaccounted for (SCHNEYER AND LEVIN, 1955). The present investigation was undertaken to determine the source of this unaccounted-for fluid. The results indicate that this fluid is attributable to three factors: secretion by small mucosal glands, condensation of fluid in the oral cavity, and unequal stimulation of the salivary glands by the various collection methods. (Supported by funds provided under contract AF 18(600)-623 with the USAF School of Aviation Medicine, Randolph Field, Tex.)

Page 163. The following abstract was omitted:

Central representation of sensory impulses in the crayfish. C. A. G. WIERSMA. *Biology Div., California Inst. of Technology, Pasadena.*

By leading off from thin fiber bundles, prepared from the commissures of the crayfish, *Cambarus clarkii*, single unit responses on sensory stimulation have been obtained. In most instances each interneuron represents a large sensory field, so that extensive convergence must occur. In many cases the sensory fields of single interneurons are spread over several to many body segments and are often non-adjacent. Each sensory area so far investigated has, however, more than a single interneuron representing it, so that divergence seems also to occur. The pattern of interneurons activated by a given sensory area is specific for that area and constant for the species. (Supported by a grant from the National Science Foundation.)

Page 213. FRANZL AND RAPPORT, *3rd line*: Instead of "in 2 fatty chains," read "and 2 fatty chains."

Page 283. SOBEL, SAMACHSON AND SLOVIK. The following acknowledgment of assistance should be added: "Supported by the Natl. Inst. for Dental Research, NIH, and by contract between the ONR, Dept. of the Navy, and the Jewish Hosp. of Brooklyn, NR 180-025."

Page 375. ORCUTT AND CRONHEIM, *line 30* should read: "falling at an approximately equal and constant rate."

Pages 476 and 583. SCHWARTZMAN *et al.*, *names of authors* should read: "GREGORY SHWARTZMAN, RENE JAHIEL* AND STANLEY M. ARONSON.*"

Page 596. E. LEONG WAY *et al.* (exhibit). *Address* should read: "*Dept. of Pharmacology, School of Medicine and College of Pharmacy, Univ. of California, San Francisco.*"

CHANGES AND ADDITIONS TO DIRECTORY LISTINGS

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